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JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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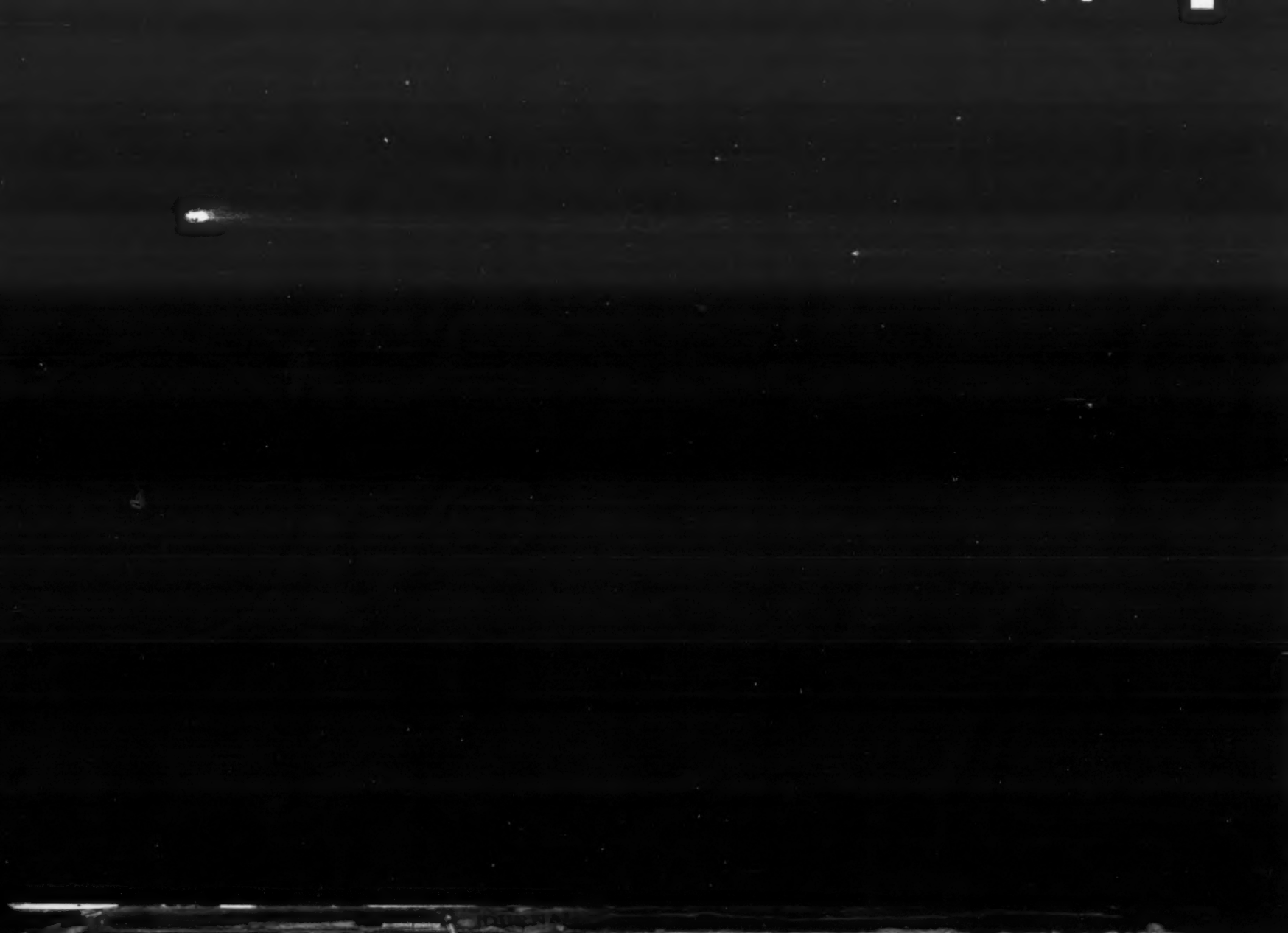

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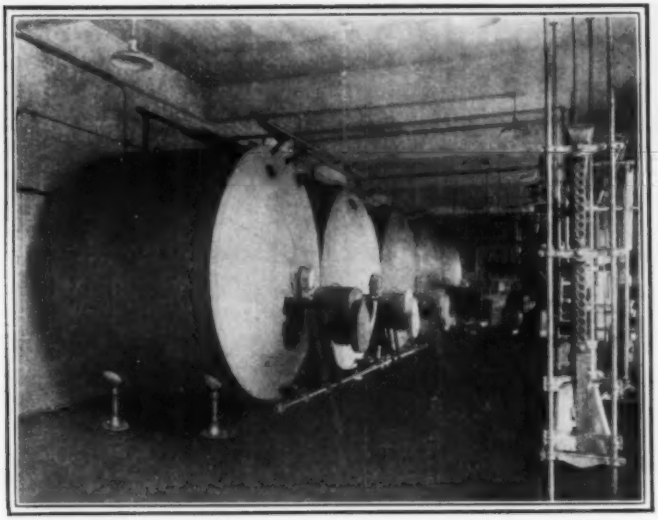

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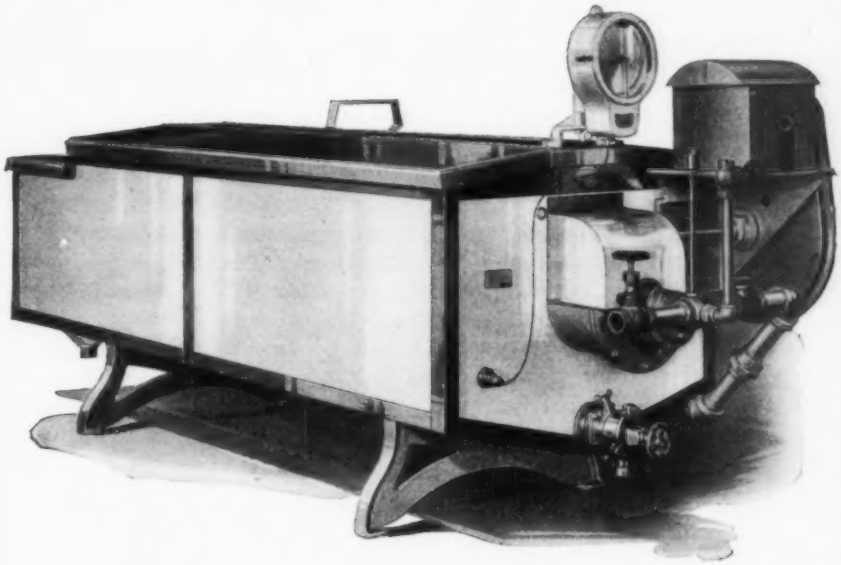

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The law since 1915 demands heat treatment for sterilization. Raw dairies and large distributing companies provide heat methods. Prior to 1919 the use of Sodium Hypochlorite for the sterilization of dairy utensils, bottles, containers, etc. was not accepted by the authorities.

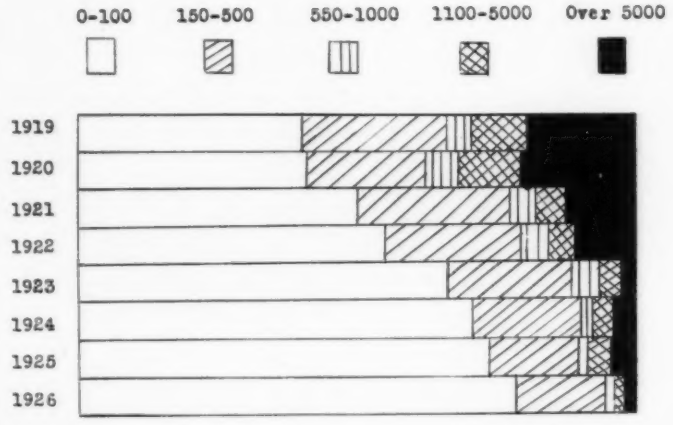
In 1920 the Health Department accepted Sodium Hypochlorite as a supplementary method for sterilizing milk utensils and later endorsed its use.

A survey of this territory made recently revealed that more than 95% of the dairymen were using Hypochlorite either as a supplemental rinse to bottles before heat or as a final rinse.

The results reported in Figure I are interesting inasmuch as the improved quality of the bottles increased year by year at about the same ratio as the use of Hypochlorite increased among the dairymen.

Change In Bacteria Counts of Bottles By Years

FIGURE I



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RELATION OF CONFORMATION AND ANATOMY OF THE DAIRY COW TO HER MILK AND BUTTERFAT PRODUCING CAPACITY

UDDER CAPACITY AND MILK SECRETION*

W. W. SWETT[1]

Dairy Cattle Breeding Investigations, Bureau of Dairy Industry, United States Department of Agriculture, Washington, D. C.

Although the mammary gland is one of the most important parts of the dairy cow, its internal anatomy, its capacity, and its performance are none too well understood. In much of the literature on dairy type or conformation, comment on these points is for some reason omitted. References which are available are not by any means in close agreement.

Concerning the general structure of the mammary gland Sisson (12) is perhaps most specific, yet he does not comment on its capacity or the milk-secreting process. Plumb (10) discusses udder structure only in a general way and states that no such irregularity of form is presented by any other anatomical part of the domestic animal as by the mammary gland of the dairy cow. Wing (13) gives a general discussion of the glandular make-up. Curtis (3) states that the size of the udder is indicative of capacity for milk production just as the size of the barrel is indicative of the capacity for food consumption. Others, in discussing dairy type, have given a more or less general and superficial description of the mammary gland.

A few have presented more definite statements concerning the capacity of the mammary gland. Isaachsen (7) is specific on this point and, in referring to cows producing 5 to 6 kilos of milk at one milking, says that "no udder contains so much." He continues by stating, "In our animals, the maximum capacity is

* Received for publication October 25, 1926.

[1] Acknowledgment is made to T. E. Woodward and Dr. F. W. Miller for helpful suggestions and assistance in the conduct of this experiment; and to Dr. L. A. Rogers for determination of milk analyses.

about 3.5 kilos or a little more, and 2 to 2.5 kilos must be formed in these animals at the moment of milking." Marshall (8) refers to calculations showing that "the udders of a cow could not contain the quantity of milk which can be obtained from them at one milking." According to Woods (14), "The flow of milk at the time of milking is usually much greater than the capacity of the milk cistern, but this is readily accounted for, as the irritation of the nerves causes the contraction of the wall of the glands and milk ducts." Scott (11) is quoted as follows:

> The reservoir or milk cistern seldom holds more than half a pint of milk. There is only one of these reservoirs in each quarter, so the combined volume of milk in the four cisterns is only two pints. This is the amount of milk which is to be found in the average dairy cow's udder at any one time.
>
> If you could look inside an udder just before milking time you would find the milk cisterns and ducts distended with milk which, as I have already mentioned, would only be about one-half pint in each quarter.

Gaines (5), on the other hand, presents data showing that (*a*) the capacity of the mammary gland of the goat is greater than the volume of milk drawn at one time; (*b*) the udder shrinks in volume during milking to nearly the same extent as the volume of the milk drawn; and (*c*) practically all the milk drawn is present as such in the gland at the beginning of milking.

Those contending that the capacity of the gland is decidedly too small to contain the quantity of milk secured at one milking, assume that milk secretion is greatly accelerated during the act of milking. Many of the exponents of this view claim that nearly all the milk, except approximately one-half pint contained in each cistern, is actually secreted while the milk is being drawn. Gaines (5) and Woods (14), however, are of the opinion that milk secretion is a continuous process. Gaines (5) comments on the work of Eckhard and on that of Goltz and Ewald, which indicates that the mammary gland is not under nervous control. McKenzie (9) also concludes that the mammary gland is not under the direct influence of the nervous system. Woods (14) contends that the manipulation of the teats and udder stimulates

the nerves, causing a muscular contraction and expression of the milk. Gaines (5) concludes that nursing or milking excites a reflex contraction of the gland and musculature, with consequent expression of milk.

Regarding the process of milk formation there is some variance in opinion. Bitting (2) outlines a theory supported by many, which is virtually that in the secretion of milk the water serum and salts are separated from the blood, and that a fatty degeneration of the cells lining the alveolar cavities produces the fat globules as the degeneration product and the casein as the undegenerated portion of the cells. He quotes one Smith as follows: "The process of milk secretion may, therefore, be regarded as a process of metabolism of the epithelial cells, which undergo decomposition, and discharge the resulting products into the excretory ducts." Bertkau (1), on the contrary, states that milk formation is a true secretory process and in no manner associated with a total or even partial necrosis of the secreting epithelium. The views of Disselhorst (4) regarding the formation of milk fat are in close agreement.

In spite of references expressing views to the contrary, the belief appears to persist rather generally among teachers and other professional men in dairy cattle and veterinary work that the capacity for storing milk in a cow's udder is not more than a half pint to each quarter. It is held that since the capacity is limited, the quantity of milk produced at any one milking could not be stored or contained in the udder, and that the greater part of the milk is secreted during the process of milking, the rapid acceleration in secretion being the result of a nervous reaction stimulated by the manipulation of the udder and teats.

The distention of the udder just before milking is a common observation. Adherents to the belief that milk secretion takes place chiefly during the act of milking have explained this distention on the theory that immediately before the milk is drawn the udder is gorged with the blood carrying the materials from which the milk constituents are to be made. Both the arterial and venous systems of several udders have been filled after their removal from the body and their total capacity has been found

to be very small. The capacity of the circulatory system within the udder therefore could hardly be an important quantitative factor in causing this distention.

The study now being conducted by the writer to determine the relation of the conformation and anatomy of the dairy cow to her milk and butterfat producing capacity has been developed to include an extensive consideration of the mammary gland. Udders are studied comparatively and in relation to their producing abilities as shown by production records. In preparing an udder for one of the phases of the study, formalin is injected into it through the teats with a milking tube attached to a pump. In filling the udder, pumping is continued until it is full and firm. It is undoubtedly distended to a greater extent than it would normally be immediately before milking at the same stage of lactation, but the pumping is stopped before pressure is sufficient to injure the tissue. The force of injection is against gravity, against the pressure of the air within the udder, and requires forcing the fluid into the fine ducts.

It has been shown that opinions differ widely concerning the capacity of the mammary gland of the cow. Udder capacity as herein discussed is understood to mean the storage space within its secretory system. It is determined by injecting formalin through the teats and measuring the quantity retained within the secretory system. In recent tests the quantity of formalin injected into the udder through the teats has been found greatly in excess of the amount it has generally been supposed the udder would hold. Data for five udders are presented in table 1. No. 245 was a hard, fleshy, fibrous udder. The quantity of formaldehyde injected into it, while not measured with precision, was between 3 and 3½ gallons, or approximately 12,000 cc. This udder had been lactating six weeks but had been badly infected. Such a condition supposedly would tend to reduce its storage capacity. No. 221 was a particularly harsh, coarse, and fibrous type of gland and had been lactating twelve weeks. It also had been infected and was secreting only a small quantity. No. 243 had been nonlactating for twelve months. It was of the meaty type but shrunken in size. No. 459 was loose and yielding and

had been lactating three months, following a premature parturition. No. 292 was a reasonably loose and mellow udder from a junior two-year old, forty-three days advanced in lactation. It appears that an udder capacity of from 3 to 5 gallons is not uncommon. For any who might be accustomed to thinking of udder capacity as the quantity of milk produced in two or more milkings, during a period of twenty-four hours, it should be mentioned that capacities herein given refer to single fillings of the udder.

An effort has recently been made to determine whether the milk in a cow's udder is secreted continuously or chiefly during

TABLE 1

Capacity of the secretory system of the mammary gland

NUMBER OF COW	BREED	PORTION OF UDDER FILLED	AMOUNT OF FORMALIN USED	CAPACITY OF SECRETORY SYSTEM	EQUIVALENT IN MILK
			cc.	*cc.*	*lb.*
245	Holstein	4 quarters	12,000‡	12,000‡	27.26
221	Holstein	4 quarters	13,000	13,000	29.53
243*	Holstein	2 right quarters	4,700	9,400	21.36
459	Jersey	2 right quarters	6,200	12,400	28.17
292†	Holstein	2 right quarters	10,200	20,400	46.35
Average					30.53

* Nonlactating.
† Fresh heifer.
‡ Approximate.

the few minutes required for the milking process. For the first test an eight-year-old Jersey, no. 459, was selected. Her only official production record, made at the age of two years one month, amounted to 10,153 pounds of milk and 536 pounds of butterfat. Her last calving was on January 29, 1926, when twins were born prematurely. Since that time her milk production had been comparatively low. During the seven-day period from April 16 to 22 inclusive, she averaged 13.91 pounds of milk when milked twice daily. Commencing on April 23, she was milked each morning at 10 o'clock. Each subsequent milking, therefore, represented the secretion of twenty-four hours. The average

to be very small. The capacity of the circulatory system within the udder therefore could hardly be an important quantitative factor in causing this distention.

The study now being conducted by the writer to determine the relation of the conformation and anatomy of the dairy cow to her milk and butterfat producing capacity has been developed to include an extensive consideration of the mammary gland. Udders are studied comparatively and in relation to their producing abilities as shown by production records. In preparing an udder for one of the phases of the study, formalin is injected into it through the teats with a milking tube attached to a pump. In filling the udder, pumping is continued until it is full and firm. It is undoubtedly distended to a greater extent than it would normally be immediately before milking at the same stage of lactation, but the pumping is stopped before pressure is sufficient to injure the tissue. The force of injection is against gravity, against the pressure of the air within the udder, and requires forcing the fluid into the fine ducts.

It has been shown that opinions differ widely concerning the capacity of the mammary gland of the cow. Udder capacity as herein discussed is understood to mean the storage space within its secretory system. It is determined by injecting formalin through the teats and measuring the quantity retained within the secretory system. In recent tests the quantity of formalin injected into the udder through the teats has been found greatly in excess of the amount it has generally been supposed the udder would hold. Data for five udders are presented in table 1. No. 245 was a hard, fleshy, fibrous udder. The quantity of formaldehyde injected into it, while not measured with precision, was between 3 and 3½ gallons, or approximately 12,000 cc. This udder had been lactating six weeks but had been badly infected. Such a condition supposedly would tend to reduce its storage capacity. No. 221 was a particularly harsh, coarse, and fibrous type of gland and had been lactating twelve weeks. It also had been infected and was secreting only a small quantity. No. 243 had been nonlactating for twelve months. It was of the meaty type but shrunken in size. No. 459 was loose and yielding and

had been lactating three months, following a premature parturition. No. 292 was a reasonably loose and mellow udder from a junior two-year old, forty-three days advanced in lactation. It appears that an udder capacity of from 3 to 5 gallons is not uncommon. For any who might be accustomed to thinking of udder capacity as the quantity of milk produced in two or more milkings, during a period of twenty-four hours, it should be mentioned that capacities herein given refer to single fillings of the udder.

An effort has recently been made to determine whether the milk in a cow's udder is secreted continuously or chiefly during

TABLE 1

Capacity of the secretory system of the mammary gland

NUMBER OF COW	BREED	PORTION OF UDDER FILLED	AMOUNT OF FORMALIN USED	CAPACITY OF SECRETORY SYSTEM	EQUIVALENT IN MILK
			cc.	*cc.*	*lb.*
245	Holstein	4 quarters	12,000‡	12,000‡	27.26
221	Holstein	4 quarters	13,000	13,000	29.53
243*	Holstein	2 right quarters	4,700	9,400	21.36
459	Jersey	2 right quarters	6,200	12,400	28.17
292†	Holstein	2 right quarters	10,200	20,400	46.35
Average					30.53

* Nonlactating.
† Fresh heifer.
‡ Approximate.

the few minutes required for the milking process. For the first test an eight-year-old Jersey, no. 459, was selected. Her only official production record, made at the age of two years one month, amounted to 10,153 pounds of milk and 536 pounds of butterfat. Her last calving was on January 29, 1926, when twins were born prematurely. Since that time her milk production had been comparatively low. During the seven-day period from April 16 to 22 inclusive, she averaged 13.91 pounds of milk when milked twice daily. Commencing on April 23, she was milked each morning at 10 o'clock. Each subsequent milking, therefore, represented the secretion of twenty-four hours. The average

production on the three successive days was 12.07 pounds. During this three-day period of adjustment and on the morning of April 27, her feed was not altered in kind or quantity. She finished eating each morning at approximately 6:30 o'clock. Until the afternoon of April 26, she was turned out with the herd for water. The quantity drunk was not measured. In order that the intake of both feed and water might be determined, water was offered her in a bucket on the afternoon of April 26 and on the morning of April 27. As she was not accustomed to drinking from a bucket, the water intake for that period may have been less than for previous days. It amounted to 24 pounds, all of which was drunk on the morning of April 27. A decrease in water intake presumably would tend to reduce the milk secretion for that day.

At ten o'clock on the morning of April 27, she was killed and immediately hoisted for bleeding. As soon as bleeding was complete she was lowered to the floor. The udder, together with an area of the skin extending from about 8 inches anterior to the front attachment to about 8 inches posterior to the rear attachment and from thigh to thigh, was then removed in such a manner that the gland tissue was not cut or injured. It was immediately attached to a specially designed iron frame, the position of the udder being adjusted until it was approximately natural for a standing cow. One hour elapsed between the killing of the animal and the completion of adjustment of the udder on the frame.

At eleven o'clock, twenty-five hours after the previous milking, the milk was drawn into a bucket in the usual manner and with approximately the usual ease and rapidity. The quantity of milk obtained from the udder thus severed and suspended was 9.2 pounds. The udder was permitted to hang in the same position for four hours until 3 p.m., when a full pint of milk (1.07 pounds) was drawn with comparative ease. A total of 10.27 pounds, therefore, was drawn from the udder after all body connections had been severed. Since the blood and lymph circulation, the nervous system, and all other body connections were severed before the milk was drawn, there could have been only a

remote possibility for milk to be formed during the milking process as a result either of nervous or muscular action stimulated by the udder manipulation. It is obvious, therefore, that more than 85 per cent of the twenty-four-hour milk production of this cow, as based on an average of the three previous days, was secreted and stored within the gland at the time she was slaughtered.

Samples of each of the two milkings immediately preceding death and each of the two portions of the 10.27 pounds of milk drawn after death were preserved for analysis. The analyses appear in table 2.

TABLE 2

Analyses of milk from no. 459

	10 A. M. APRIL 25, 1926—FORTY-EIGHT HOURS BEFORE DEATH	10 A.M. APRIL 26, 1926—TWENTY-FOUR HOURS BEFORE DEATH	11 A. M. APRIL 27, 1926— FIRST POST-MORTEM MILKING	3 P. M. APRIL 27,1926—SECOND POST-MORTEM MILKING
Quantity (pounds)	11.00	12.10	9.20	1.07
	per cent	*per cent*	*per cent*	*per cent*
Total solids	14.71	14.65	13.88	10.23
Fat	5.75	5.73	4.50	1.60
Solids-not-fat	8.96	8.92	9.38	8.63
Ash	0.832	0.831	0.877	0.939
Casein	2.88	2.81	3.03	2.88
Albumin	0.75	0.77	0.76	0.75
Sugar	4.498	4.509	4.713	4.061

Immediately after the post-mortem milking had been completed, the right half of the udder was filled with formaldehyde and prepared for the study of its gross structure. The left half was removed by an incision just to the left of the median septum and was preserved for histological analysis. The external appearance of the udder before its removal from the cow is shown in figure 1. Figure 2 shows a vertical transverse section through the right rear quarter and illustrates the internal structure of the gland. The tissue is relatively open in structure with comparatively large cisterns. The fact that these conditions were definitely recorded in the observations made through an entire lactation period many months previously lends value to 2500

to 3000 sets of observations that have been made according to the same plan on other living cows.

A second test was conducted on no. 292, a junior two-year-old Holstein, forty-three days advanced in her first lactation period. Since her production was relatively high she was continued on two milkings daily. From the afternoon of July 1 to the morning of July 8, 1926, she averaged 43.36 pounds of milk daily. During this period the average quantity produced at the morning milking was 21.69 pounds. She was milked regularly at 9:30 a.m. and

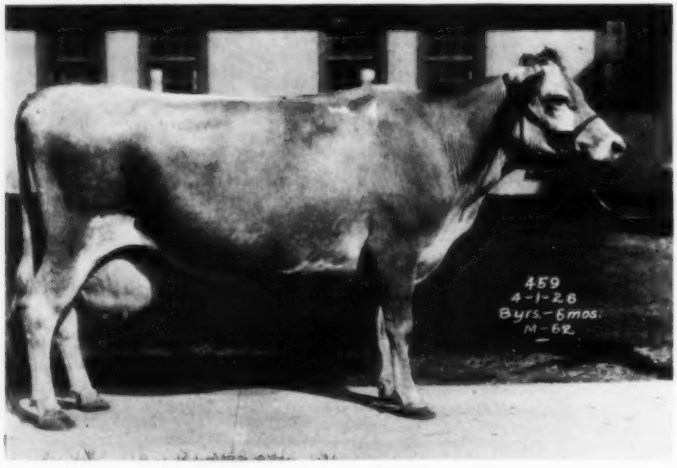

FIG. 1. THE UDDER OF NO. 459 BEFORE ITS REMOVAL

9:30 p.m., each milking representing the production of twelve hours. The kind and quantity of feed and the time of feeding and watering were maintained as nearly as possible without change.

She was killed at 9:30 a.m. July 9, 1926, twelve hours after the last milking. It was necessary to lead her a considerable distance for slaughter and although this was done quietly, she became somewhat excited immediately before death. The effect of this excitement of course is not known. The procedure in bleeding and in removing the udder was identical with that followed in the previous test.

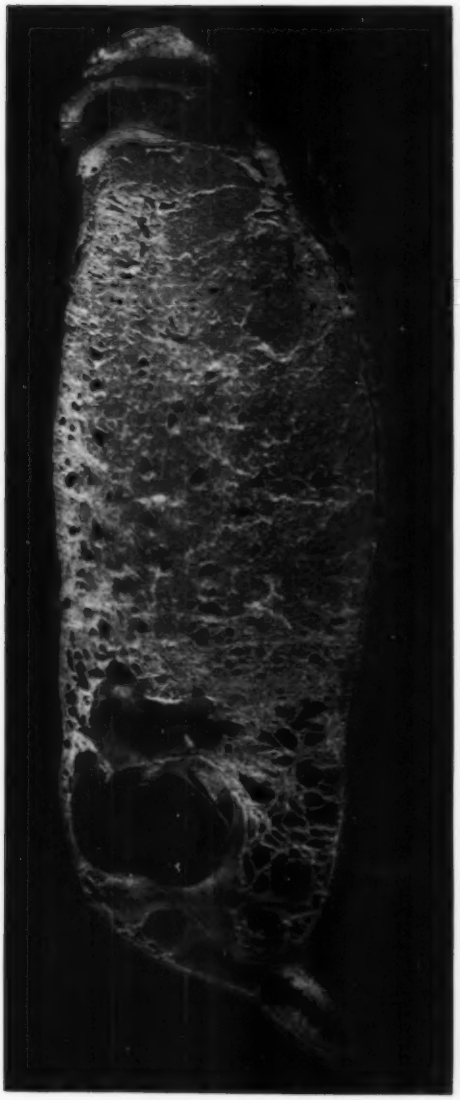

FIG. 2. A VERTICAL TRANSVERSE SECTION THROUGH THE RIGHT REAR QUARTER OF NO. 459'S UDDER

Milking was commenced at 10:20 a.m. The milk was drawn much more slowly than in the post-mortem milking of no. 459. The udder was massaged as it was milked. The quantity secured was 7.7 pounds. Four hours later, at 2:20 p.m., the udder was again milked and 2.9 pounds was drawn, making a total of 10.6 pounds after death. When the udder was cut open a short time later the milk gushed from the incision. An abundant quantity of milk appeared to be present in the udder, but it was not so readily drawn as in the previous test. The temperature of the milk was taken at about the middle and near the end of the first post-mortem milking and varied from 96° to 93°F. The tem-

TABLE 3

Analyses of milk from no. 262

	9:30 A. M. JULY 7, 1926—FORTY-EIGHT HOURS BEFORE DEATH	9:30 A.M. JULY 8, 1926—TWENTY-FOUR HOURS BEFORE DEATH	10:20 A. M. JULY 9, 1926—FIRST POST MORTEM MILKING	2:20 P. M. JULY 9, 1926—SECOND POST-MORTEM MILKING
Quantity (pounds)	24.60	22.00	7.70	2.90
	per cent	*per cent*	*per cent*	*per cent*
Total solids	10.88	10.77	9.61	7.88
Fat	2.95	2.90	1.28	0.70
Solids-not-fat	7.93	7.87	8.33	7.18
Ash	0.757	0.736	0.778	0.818
Casein	2.092	2.021	2.023	1.766
Albumin	0.281	0.313	0.326	0.324
Sugar	4.800	4.800	5.203	4.272

perature of the milk of the second post-mortem milking was 86°F. Samples of the morning milkings of July 7 and July 8 and of both post-mortem milkings were preserved, and their analyses appear in table 3. The udder was treated in the manner described for no. 459. The right half was preserved and sectioned. The external appearance of the udder before its removal from the cow is illustrated in figure 3, and a vertical transverse section through the right rear quarter is shown in figure 4. Reference to table 1 will show the udder capacity of both nos. 459 and 292.

There is a difference between the composition of the ante-mortem and post-mortem milkings which deserves brief comment. This difference is remarkably similar in the milk from

both cows. The fourth sample, which is taken from the final post-mortem milking, is the one differing most from the ante-mortem product. In each case the final post-mortem milking shows an increase in ash and a decrease in sugar when compared with that drawn before death. In the case of no. 292, this fourth sample also shows a low casein content. The outstanding difference, however, is in the fat content, which is greatly lowered in both of the post-mortem milkings and largely responsible for the reduction in total solids in these samples.

FIG. 3. THE UDDER OF NO. 292 BEFORE ITS REMOVAL

If it were true that the fat in milk is a decomposition product of the cells, and if this process actually takes place chiefly during the act of milking, this low fat content might be accounted for. It would seem, however, that if secreted chiefly during the milking of the cow, milk taken from a gland entirely removed from the body and in which all activity apparently had ceased would have a fat content even lower than that found. If the high fat content normally found in strippings and in last milk were due to gravitation, these post-mortem samples, particularly the last one, should be higher in fat content than the others.

FIG. 4. A VERTICAL TRANSVERSE SECTION THROUGH THE RIGHT REAR QUARTER OF NO. 292'S UDDER

If, on the contrary, milk secretion is a continuous process, with all the constituents secreted simultaneously, the diminished fat content in the post-mortem samples must be accounted for in some other way. Hunziker (6) gives the melting point of mixed butterfat as 90° to 99°F. At lower temperatures the fat presumably would become less fluid. No attempt was made in either case to maintain body temperature in the udder after its removal. The temperature of the post-mortem milk from no. 459 was not determined. For no. 292 it was 96° to 93°F. for the first post-mortem milking and 86°F. for the second. The change in consistency of the fat as a result of the lowered temperature of the gland might cause the retention of a large proportion of the fat in the smaller ducts and easily account for the abnormally low fat content in the post-mortem milkings.

The first two tests reported would indicate that:

1. Milk secretion is to a considerable extent a continuous process.

2. A large proportion of the milk secured at any milking is collected and stored within the gland before the milking process is commenced.

3. The internal capacity of a lactating cow's udder appears to be greater than the volume of the milk secreted. The two udders on which results are here reported were not exceptionally large, yet one of them indicates an internal space sufficient to hold as much as 45 pounds of milk.

4. The liberation of milk is not entirely dependent either upon a nervous or a mechanical stimulation or upon internal muscular contraction, since the act of milking was in both cases performed after all body connections had been severed. While in the post-mortem milking, no. 459 readily liberated about 85 per cent of the amount produced in like periods before death, no. 292 yielded only about 49 per cent. The gushing forth of milk from an incision subsequently made in the udder of no. 292 indicates that in her case the milking was only partially complete. Apparently a relatively high proportion of her average milk production for a like period was present in the udder at the time of death. The reason for the greater difficulty in removing the milk in her case

is not definitely understood. It would seem, however, that muscular contraction, or in this case, *rigor mortis*, may have hindered rather than aided the removal of the milk.

5. The outstanding abnormality in the milk drawn after death is its low fat content. This might be accounted for on the theory that the fat globules were retained within the ducts as a result of the lowered temperature after death of the gland and of the milk contained within it.

The results of these tests are not conclusive. Additional tests will be conducted and the results published as material is made available.

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A STUDY OF THE PROTEOLYTIC ACTION OF CERTAIN SPECIFIC ORGANISMS ON MILK PROTEINS IN MILK AND SYNTHETIC BUTTER*

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Considerable work has already been done by a number of investigators on bacterial metabolism including yeasts and molds, with a view of obtaining a quantitative relation of the action of specific organisms when grown in different media. These investigations were generally limited to pathogenic organisms. However, only in a few instances has milk been used as a media. It is evident that in milk we have a natural product, composed of proteins, carbohydrate and mineral salts, well adapted for bacterial growth, the study of which is of prime importance to the dairy industry. It is well known that the composition of the media influences the growth and development of bacteria. For the study of pathogenic organisms the media is usually composed of nitrogenous materials and an adequate supply of mineral salts. It has been found that when these organisms are grown in a media containing some utilizable carbohydrate, that the chemical changes produced in the media are quite different than when grown in media containing no carbohydrate. Notably, at least in some organisms, no proteolytic enzymes are formed (1) (2) (3).

In our investigation we have confined our study of the proteolytic action of certain specific organisms usually found in milk and manufactured dairy products. This involves the study of bacterial metabolism or cellular activity. The protein requirements and the energy requirements must be supplied by the food material in the media. There is sufficient evidence that the

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cellular activity of all organisms are intimately associated with enzymes, and it is by this means that chemical changes are brought about, which gives us a measure of the rôle played by organisms in the decomposition of the food material in the media.

The proteolytic enzymes of organisms may be classified into two well defined groups—the peptic group of enzymes which act and are accelerated in acid media; the tryptic group which act and are accelerated in an alkalin media. Some organisms secrete both groups. In fact, no enzyme has yet been found or isolated which is specific in action as a unit rather than a mixture. This fact of a mixture of the two groups of enzymes is unmistakably shown in our previous work on the enzymes of B. panis migula (5). In this investitation it was found that when the enzyme acted in a 0.2 per cent acid solution, 5.1 per cent of the total nitrogen apeared as amino acids; when acting in a 0.2 per cent basic solution 14.00 per cent of amino acids was formed both solutions being digested for thirty-three days. However, when digested in neutral solution, 25.35 per cent of amino acids was formed. The characteristic action of both the tryptic and peptic enzymes was clearly manifested in this experiment (5).

In addition to the proteolytic enzymes, there is usually accompanied sugar splitting enzymes as well as fat splitting enzymes. In this investigation we are concerned only with the protein splitting enzymes. In dairy products we have both the protein and sugar as lactose. As lactose is hydrolyzed by enzymes of a zymase character, the acid prouuced will influence to a great degree the soluble or exo enzyme production of the organism (1), (2), (3), and consequently proteolytic action is retarded. There is a maximum degree of acidity for the growth and development of the organisms themselves. Unless soluble proteolytic enzymes are formed during the process of bacterial growth, no proteolytic action will take place even though the media is neutralized. In our study of B. panis migula when grown in sterile milk we found at the end of thirty days 26.40 per cent of total nitrogen appearing as amino acids. Fifteen days longer incubation brought about no increase neither in

ammonia nor amino acids, the acidity of the milk being at the end of 45 days, 0.540 per cent. The high acidity inhibited the action of the enzymes and the functioning of the organism.

The production of ammonia by means of bacterial action is a measure of bacterial metabolism, taking place within the organism. In the utilization of protein, the molecules are hydrolized to simpler compounds, peptones, amino acids, etc. When these compounds are utilized for energy production, deaminization takes place and the ammonia is the end product and appears in the media. The production of ammonia is no indication of the changes taking place exterior to the organism. It is therefore evident, to gain a more definite idea and interpretation of protein decomposition, it is necessary to secure data of the amino acids, ammonia and peptones produced by proteolytic organisms.

The determination of titratable acidity is an indirect measurement of the dissociated acidity or hydrogen-ion concentration, if this is based on a given acid and for a media of constant composition.

In this study of the proteolytic action of organisms we have limited our data to what seemed to us the most important cleavage products and to show to what extent, under certain conditions, these changes have taken place in proteins of milk and synthetic butter. Owing to the separation of the proteins during sterilization it was found impossible to sterilize butter, and bring it to the original homogeneous condition.

It is known that it is difficult to account for the numerous changes caused by the organisms; nor is it possible to solve complex problems with the sum total action of these organisms. If we can trace the deterioration of groups of organisms, some good has been accomplished. It seemed therefore desirable to eliminate this complexity and use specific organism in sterile media.

BACTERIOLOGICAL WORK

Milk. Two hundred fifty cubic centimeters of fresh skimmilk were sterilized in a 500 cc. Erlenmeyer flask in an autoclave at 15 pounds for thirty minutes. In the sterile milk is inoculated 1 cc.

of a twenty-four-hour culture of the organism to be studied. Incubation temperature was room temperature (20° to 21°C.) Analyses were made each fifteen days.

Synthetic butter. Six hundred-gram lots of synthetic butter were prepared as follows: Pure butterfat was obtained by melting creamery butter at a temperature between 60° to 70°C. When the water and curd were well separated by gravity the clear fat was filtered and sterilized in definite amounts. Fifty grams of skimmilk with an acid reaction of 0.17 per cent were introduced into a 500 cc. Erlenmeyer flask and sterilized in an autoclave at 15 pounds pressure for thirty minutes. To this sterile skimmilk a pure twenty-four-hour culture of the organism to be studied was introduced and incubated for twenty-four hours at 37°C. The sterile fat, was introduced into the inoculated skimmilk. The flask was gradually cooled in ice water, and gently shaken so as to insure a homogeneous consistency. The blank was prepared in like manner except that it was not inoculated. The synthetic butter had approximately the following composition: protein 0.6 per cent, water 14.6 per cent, lactose 1.0 per cent, and fat 84.0 per cent. The synthetic butter was held at room temperature and at 0° to 4°C. The synthetic butter held at room temperature was submitted to analysis each 15 days while that at 0° to 4°C. was analyzed every thirty days.

The organisms used were obtained from reliable sources and rechecked in order to determine their purity and nomenclature. Twenty-four-hour cultures were made into sterile skimmilk for three successive days in order to vitalize the organism. The following organisms were used:

Bacillus panis
Pseudonionas viscosa
Proteus vulgaris Hauser
Bacillus megatherium
Bacillus amylobacter
Bacillus mesentericus Flugge
Streptococcus lactis Lester
Escherichia coli
Bacillus mycoides
Achromobacter liquefaciens
Bacillus subtilis
Aerobacter aerogenes
Escherichia ichthyosmia

The above nomenclature is according to Bergey's Manual of Determinative Bacteriology.

CHEMICAL WORK

The chemical work in this investigation consisted in determining the titratable acidity, nitrogen compounds not precipitated by phosphotungstic acid and nitrogen compounds not precipitated in a saturated solution of zinc sulphate. The peptones represent the difference between the nitrogen compounds not precipitated by zinc sulphate and phosphotungstic acid. It is generally agreed that phosphotungstic acid precipitates all the higher complexes including the diamino acids, but does not precipitate the mon-amino acids. The nitrogen compounds not precipitated by phosphotungstic acid are frequently considered as representing only amino acids, which should be accepted as relatively correct. We have found in previous work[1] the amino acids as determined by the Van Slyke method are lower, but with practically a constant difference. The data represent more correctly, representative well defined groups of protein compounds and show the progress of protein hydrolysis.

The ammonia was determined by the Folin aeration method. Two hundred cubic centimeters of the milk was used and after aeration this was neutralized and used for the following determinations. To 10 cc. of the aerated milk sufficient 20 per cent sulphuric acid was added to make the acidity 5 per cent. To this a 5 per cent solution of phosphotungstic acid solution was added to cause complete precipitation of the proteins. It was then set aside at room temperature for twelve hours, then filtered, and the nitrogen determined in the filtrate by using the Kjeldahl method.

To 10 cc. of the aerated milk dilute sulphuric acid was added to give a distinct acid reaction to methyl red. This was saturated with pure zinc sulphate by slightly warming and left standing at room temperature for twelve hours. This was then brought up to 30 cc. by adding a saturated solution of zinc sulphate. This was filtered and 15 cc. of the filtrate was used for the nitrogen determination. The peptones were estimated by subtracting the nitrogen not precipitated by phosphotungstic acid from the nitrogen not precipitated by a saturated solution of zinc sulphate. The results in tables were based on the total nitrogen.

[1] Unpublished data.

Two hundred and fifty grams of butter were weighed into a beaker, warmed and transferred to a 500 cc. flask. About 100

TABLE 1

Acidity produced by the respective specific organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CONTROL
	per cent	*per cent*	*per cent*	*per cent*
panis	0.324	0.409	0.450	0.243
viscosa	0.170	0.103	0.108	
vulgaris	0.170	0.090	0.108	
megatherium	0.464	0.504	0.414	
amylobacter	0.310	0.378	0.378	
coli	0.67	0.77	0.78	
mesentericus	0.74	0.31	0.56	
lactis	0.925	0.70	0.91	
liquefaciens	0.396	0.740	0.675	
mycoides	0.19	0.49	1.09	
subtilis	0.378	0.387	0.540	
aerogenes	0.634	0.693	0.727	
ichthyosma	0.531	0.414	0.414	

TABLE 2

Ammonia produced by the specific organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	0.50	1.29	3.91	0.32
viscosa	0.30	0.51	1.25	
vulgaris	0.42	0.51	1.14	
megatherium	1.84	4.13	11.65	
amylobacter	1.47	2.61	5.40	
coli	0.10	0.13	0.97	
mesentericus	0.30	1.88	4.05	0.34
lactis	0.13	0.24	0.38	
liquefaciens	4.32	7.01	7.69	
mycoides	0.73	1.43	3.96	
subtilis	2.42	5.67	7.27	0.35
aerogenes	1.88		2.57	
ichthyosma	9.03	20.16	25.68	

cc. portions of water at 50° to 60°C. was poured into the beaker, and the mixture transferred to the flask after standing to permit

the separation of the aqueous portion from the fat; the separated water and proteins were pipetted into a 250 cc. graduated flask.

TABLE 3

Nitrogen compounds not precipitated by phosphotungstic acid produced by the respective organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	7.10	8.93	17.00	3.47
viscosa	4.04	4.26	4.49	
vulgaris	3.50	3.52	8.74	
megatherium	12.18	12.56	27.66	
amylobacter	7.51	12.11	18.83	
coli	3.75	3.61	4.06	3.35
mesentericus	8.65	13.65	20.00	5.00
lactis	4.40	5.33		5.15
liquefaciens	16.14	31.63	31.86	4.93
mycoides	5.38	9.42	19.99	4.93
subtilis	8.61	17.57	31.13	3.41
aerogenes	3.01	3.87	8.32	3.42
ichthyosma	33.20	51.90	53.01	3.02

TABLE 4

Nitrogen compounds not precipitated by a saturated solution of zinc sulphate produced by the respective organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	13.46	15.38	26.92	4.60
viscosa	6.51	6.56	6.75	4.60
vulgaris	4.60		8.97	4.60
megatherium	16.37	15.00	33.62	4.60
amylobacter	11.66	18.84	29.16	4.60
coli	5.86	6.76	6.31	4.60
mesentericus	17.30	31.87	41.75	7.28
lactis	6.53	8.40		7.28
liquefaciens	22.43	38.70	50.25	7.28
mycoides	10.77	22.43	39.04	7.28
subtilis	22.36	22.36	32.58	6.55
aerogenes	6.37		10.01	6.55
ichthyosma	66.40	68.30	71.61	6.55

The process of adding warm water to the butter was repeated until 250 cc. of protein solution had been obtained. This process

TABLE 5

Nitrogen compounds as peptones produced by the respective organisms inoculated in sterile milk held at 20°C.

	15 DAYS INCUBATION	30 DAYS INCUBATION	45 DAYS INCUBATION	CHECK
	per cent	*per cent*	*per cent*	*per cent*
panis	6.36	6.45	9.92	1.13
viscosa	2.47	2.30	2.26	
vulgaris	1.10		2.03	
megatherium	4.19	5.44	5.96	
amylobacter	4.15	6.73	10.33	
coli	2.11	3.15	2.25	
mesentericus	8.65	18.22	21.75	
lactis	2.13	3.07		
liquefaciens	6.29	7.07	18.49	
mycoides	5.39	13.01	19.05	
subtilis	13.75	4.79	1.45	
aerogenes	3.36		1.69	
ichthyosma	33.20	16.40	18.60	

TABLE 6

Summary of tables 1 to 5 showing the increase of ammonia, nitrogen compounds not precipitated by phosphotungstic acid and peptones in milk by the action of specific organisms during a period of 45 days based on the total nitrogen

	AMMONIA	NITROGEN NOT PRECIPITATED BY PHOSPHO-TUNGSTIC ACID	PEPTONE
	per cent	*per cent*	*per cent*
panis	3.59	13.53	8.79
viscosa	0.93	1.02	1.13
vulgaris	0.82	5.27	0.90
megatherium	11.33	24.19	4.83
amylobacter	5.08	15.36	9.20
coli	0.65	0.71	1.12
mesentericus	3.71	15.00	20.62
lactis	0.06	0.18	1.94
liquefaciens	7.37	26.93	17.36
mycoides	3.64	15.06	17.92
subtilis	6.95	27.72	1.32
aerogenes	2.25	4.90	0.56
ichthyosma	25.36	49.99	17.47

removed more than 96 per cent of the protein. The graduated flask was set aside in a warm place until the particles of fat

had come into the neck. These were removed by means of a pipette. This solution was then treated in the same manner as the milk was treated for the determination of ammonia, amino

TABLE 7

Acidity produced by the respective specific organisms inoculated in sterile butter held at room temperature, 20°C.

	15 DAYS HELD	30 DAYS HELD	40 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	0.033	0.030	0.090	0.091	0.011
viscosa	0.025	0.032	0.198	0.201	0.011
vulgaris	0.015	0.022	0.045	0.049	0.011
megatherium	0.22	0.023	0.018	0.022	0.011
butyricus	0.013	0.011	0.162	0.185	0.011
coli	0.100	0.144	0.148	0.138	0.011
mesentericus	0.067	0.09	0.251	0.248	0.017
lactis	0.175	0.268	0 315	0.340	0.016
liquefaciens	0.109	0.126	0.121	0.101	0.016
mycoides	0.099	0.122	0.125		0.016

TABLE 8

Ammonia produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis		0.90	1.08	1.43	0.35
viscosa	0.63	1.07	2.14		0.35
vulgaris	0.65	0.79	1.00	1.04	0.35
megatherium	1.11	1.43	1.69	1.78	0.35
butyricus	1.53	2.14	2.14		0.35
coli	0.27		0.71		0.21
mesentericus	1.18		0.80	0.84	0.21
lactis	0.20		0.89	0.87	0.21
liquefaciens	1.25		1.78	2.35	0.21
mycoides	1.07			1.34	0.21

acids, peptones, etc.; in the precipitation by phosphotungstic acid and zinc sulphate, 50 cc. of the protein solution was used.

All determinations were made in duplicate.

Analyses of the inoculated milk, synthetic butter held at room temperature and synthetic butter held in cold storage at 0° to

4°C. were made at regular intervals. The milk and butter held at room temperature were analyzed every fifteen days, the butter held in cold storage every thirty days.

TABLE 9

Nitrogen compounds not precipitated by phosphotungstic acid produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	10.70	12.50	14.29	29.11	4.35
viscosa	7.05	7.50	11.61	20.80	
vulgaris	4.10	5.35	6.25	15.62	
megatherium	16.05	17.94	18.75	23.48	
butyricus	8.75	14.37	14.50	24.10	
coli	3.66	4.73	11.52	11.50	
mesentericus	11.78	11.70	17.68	20.18	
lactis*	3.75	6.43			
liquefaciens	7.96	9.64	12.86	20.44	
mycoides	9.03	8.80	9.18	15.44	

* Contaminated.

TABLE 10

Nitrogen compounds not precipitated by saturated solution of zinc sulphate produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	20.53	18.75	25.80	50.00	7.58
viscosa	8.75	9.10	12.50	41.07	
vulgaris	6.61	6.70	9.82	38.21	
megatherium	31.25	32.14		41.07	
butyricus	10.00	18.75	18.00	26.24	
coli	4.46	6.00		17.49	
mesentericus	19.01	19.64	24.11	29.37	
lactis	5.80	9.64			
liquefaciens	18.30	20.00	21.25	25.70	
mycoides	16.96		18.60	27.32	

In order to render the results of this investigation of value, extra effort was made to eliminate factors interfering with the uniformity such as temperature of storage and precipitation of proteins. This gives some assurance of the relative comparableness of our results.

Tables 1 to 15 show the changes caused by the organisms studied, in sterile milk, sterile synthetic butter held at room temperature and in cold storage.

TABLE 11

Nitrogen compounds as peptones produced by the respective specific organisms inoculated in sterile butter held at 20°C.

	15 DAYS HELD	30 DAYS HELD	45 DAYS HELD	60 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	9.83	6.25	11.55	20.89	2.23
viscosa	1.70	1.60	0.89	20.27	
vulgaris	2.51	1.35	3.57	12.59	
megatherium	15.20	14.20		17.59	
butyricus	1.25	4.38	3.50	2.14	
coli	0.80	1.27		5.99	
mesentericus	7.23	8.94	7.45	9.19	
lactis	2.05	2.21		2.31	
liquefaciens	10.34	10.36	8.37	5.26	
mycoides	7.93		9.22	11.88	

TABLE 12

Summary of tables 7 to 11 showing the increase of ammonia nitrogen compounds not precipitated by phosphotungstic acid and peptones by the action of specific organisms in synthetic butter held at room temperature for 60 days

	AMMONIA	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	PEPTONES
panis	1.08	13.76	18.66
viscosa	1.79	14.45	18.04
vulgaris	0.69	10.27	10.36
megatherium	1.43	18.13	15.36
butyricus	1.79	18.75	0.09
coli	0.50	6.15	3.76
mesentericus	0.59	14.83	6.96
acidi lactis	0.66	6.35	0.08
liquefaciens	2.14	15.09	3.03
mycoides	1.13	10.09	9.65

Throughout the tables, only the specific names of organism will be used.

Comments of results from data in tables 1 to 6. The production of acidity as determined by titration of most organisms reached the maximum acidity during the first period of fifteen days.

Panis, mycoides, subtilis, and aerogenes continued to increase the acidity until the end of the third period of forty-five days. This increase was very slight during the second to the third

TABLE 13

Acidity produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS HELD	60 DAYS HELD	90 DAYS HELD	120 DAYS HELD	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	0.018	0.019	0.020	0.016	0.018
viscosis	0.027	0.030	0.026	0.049	
vulgaris	0.013	0.014	0.013	0.016	
megatherium	0.009	0.011	0.012	0.015	
butyricus	0.004	0.008	0.010	0.017	
coli	0.068	0.117	0.125	0.135	0.036
mesentericus	0.072	0.108	0.112	0.099	
lactis	0.108	0.207	0.224	0.244	
liquefaciens	0.072	0.145	0.144	0.140	
mycoides	0.063	0.126	0.185	0.135	

TABLE 14

Ammonia produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	0.252	0.193	0.320	1.34	0.192
viscosa	0.255	0.546		1.01	
vulgaris	0.42	0.319	0.757	0.757	
megatherium	0.377	0.386	0.300	0.840	
butyricus	0.184	0.512	0.462	1.17	
coli	0.420	0.580		0.588	
mesentericus	0.42	0.330	0.625	0.714	
lactis	0.310	0.170	0.312	0.357	
liquefaciens	0.425	1.010	1.00	0.89	
mycoides	0.358	1.258	1.331	1.348	

period; evidently the acidity reached its maximum at the end of thirty days incubation.

In the summary of table 6 the production of ammonia, amino acids and peptones is strikingly shown when the organisms are grown in milk as the media. All organisms producing a high

per cent of ammonia were likewise active in producing amino acids and peptones, there being some exceptions to the proportion of ammonia, amino acids and peptone. The highest per

TABLE 15

Nitrogen compounds not precipitated by pho photungstic acid produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	6.64	11.01	13.361	27.40	6.64
viscosa	7.14	6.64	8.82	19.57	
vulgaris	4.20	5.40	11.67	23.10	
megatherium	9.40	11.03	13.03	13.70	
butyricus		7.56	10.42	14.30	
coli	3.20	4.46	5.00	5.16	5.00
mesentericus	5.89	8.04	8.93	9.82	
lactis	5.35	4.50	5.80	6.24	
liquefaciens	7.50	9.28	9.47	10.71	
mycoides	6.43	9.19	9.46	9.00	

TABLE 16

Nitrogen compounds not precipitated by saturated solution of zinc sulphate by the action of the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	11.76	21 43	23.20	47.07	7 06
viscosis	8.41	8 57	9.91	30.75	
vulgaris	8.40	9.41	13.76	35.98	
megatherium	11.76	12.77	15.30	39.15	
butyricus		14.45	13.61	17.14	
coli	8.30	11.25	11.61	12.32	
mesentericus	9.20	14.82	15.35	16.07	
lactis	9.20	10.35	11.61	12.85	
liquefaciens	11.60	12.60	15.17	16.07	
mycoides	9.55	11.07	11.60	12.05	

cent of ammonia produced was that by ichthyosma. Likewise this organism produced the highest per cent of amino acids but not of peptones.

Comments of results from data in tables 7 to 12, organisms in synthetic butter held at room temperature and in tables 13 to 18, synthetic butter held in cold storage, time of holding sixty and

TABLE 17

Nitrogen compounds as peptones produced by the respective specific organisms inoculated in sterile butter held in cold storage at 0° to 4°C.

	30 DAYS STORAGE	60 DAYS STORAGE	90 DAYS STORAGE	120 DAYS STORAGE	CONTROL
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
panis	5.12	10.42	9.59	19.67	0.42
viscosis	1.27	1.93	1.09	11.18	
vulgaris	4.20	4.01	2.09	12.88	
megatherium	2.36	1.74	2.27	25.45	
butyricus		6.89	3.19	2.84	
coli	5.10	6.79	6.61	7.15	
mesentericus	3.31	6.78	6.42	6.25	
lactis	3.85	5.85	5.81	6.61	
liquefaciens	4.10	3.32	5.70	5.14	
mycoides	3.12	1.88	2.40	4.05	

TABLE 18

Summary of tables 11 to 15 showing the increase of ammonia, nitrogen, compounds not precipitated by phosphotungstic acid and peptones by the action of specific organisms in synthetic butter held in cold storage for 120 days

	AMMONIA	NITROGEN NOT PRECIPITATED BY PHOSPHOTUNGSTIC ACID	PEPTONES
	per cent	*per cent*	*per cent*
panis	1.148	20.76	19.25
viscosa	0.818	12.93	10.76
vulgaris	0.565	16.46	12.46
megatherium	0.648	7.06	25.03
butyricus	0.978	7.66	2.42
coli	0.396	0.17	6.73
mesentericus	0.523	4.82	5.83
lactis	0.165	1.24	6.19
liquefaciens	0.698	5.71	1.72
mycoides	1.156	4.00	2.63

one hundred and twenty days, respectively. In the synthetic butter there was a continued increase in the acidity of all organisms studied. S. lactis produced an acidity of 0.34 per cent during

storage of sixty days at 20°C., table 7. In the synthetic butter held at 0° to 4°F. for a period of one hundred and twenty days,

TABLE 19

A comparison of the actions of proteolytic organisms on the proteins in milk at room temperature, synthetic butter held at room temperature, 20°C. and synthetic butter held in cold storage 0° to 4°C.

ORGANISMS	NITROGEN NOT PLECIPITATED BY PHOSPHOTUNGSTIC ACID			NITROGEN AS PEPTONES		
	Milk	Synthetic butter		Milk	Synthetic butter	
	Room temperature	Room temperature	Cold storage	Room temperature	Room temperature	Cold storage
	At end of 45 days	At end of 60 days	At end of 120 days	At end of 45 days	At end of 60 days	At end of 120 days
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
panis	13.53	13.76	20.76	8.79	18.66	19.25
vulgaris	5.27	10.27	12.93	0.90	10.36	10.76
megatherium	24.19	18.13	7.66	4.83	15.36	25.03
mesentericus	15.00	14.83	4.82	20.62	6.96	5.83
liquefaciens	26.93	15.09	5.71	17.36	3.03	1.72
mycoides	15.06	10.09	4.00	17.92	9.65	2.63
Average	16.65	12.02	9.30	11.70	10.06	10.90

TABLE 20

Acidity of the inoculated milk and synthetic butters at the end of the respective periods of incubation

Tabulated for the purpose of comparing the acidity produced by these proteolytic organisms with results as shown in table 19

ORGANISMS	MILK	SYNTHETIC BUTTER	
	Room temperature	Room temperature	Cold storage
	per cent	*per cent*	*per cent*
panis	0.450	0.091	0.016
vulgaris	0.108	0.049	0.016
megatherium	0.414	0.022	0.015
mesentericus	0.560	0.248	0.099
liquefaciens	0.675	0.101	0.140
mycoides	0.540	0.125	0.135
Average	0.458	0.109	0.07

S. lactis produced an acidity of 0.244 per cent. The same relative increase in acidity followed when the butter was stored at

room temperature for sixty days as when stored at 0° to 4°C. except for B. panis migula, Proteus vulgaris and B. megatherium which produced no acidity as shown by the control.

The production of ammonia by the respective organisms is shown in tables 8 and 14. In each instance there was a gradual increase of ammonia by the organisms whether butter was held at room temperature or in cold storage and this quite uniformly, there being few exceptions. It is to be noted that B. panis migula and B. mycoides produced as much ammonia when butter was held for sixty days at room temperature as when held at 0° to 4°C. for one hundred and twenty days.

That there was a continual increase of both the amino acids and peptones during storage is clearly shown in tables 9 and 11 for butter stored at room temperature, and in tables 15 and 17 for butter stored at 0° to 4°C., there being few exceptions. It is especially to be noted that organisms active in ammonia productions were also correspondingly active in hydrolizing the proteins to amino acids and peptones.

In this investigation the hydrolysis of milk proteins in milk and synthetic butters into simple compounds during a period of forty-five, sixty and one hundred and twenty days is shown in table 19. The organisms are selected for the reason that they were used in both the milk and synthetic butters.

DISCUSSION

In the study of the action of proteolytic organisms we are concerned with end and cleavage products formed. The composition of the media at the beginning and the chemical changes produced during the investigation furnishes evidence as to the character and progress of the changes brought about by organisms used.

It is to be noted that all organisms producing ammonia hydrolized a corresponding amount of proteins into amino acids and peptones. While there is considerable variation in the ratio of these cleavage products for different organisms, this variation may be accounted for if we consider the proteolytic action of

enzymes. In our comparison of the changes brought about by six proteolytic organisms during the respective periods of holding, we would, according to the law of mass action, expect a much greater per cent of protein in milk hydrolized than in the synthetic butter, there being in milk 3 per cent protein and in the synthetic butter, 0.6 per cent. Several important factors are involved in explanation of this difference. It has been shown by Sears, Jones and Kendall and co-workers that in presence of a utilizable carbohydrate, as dextrose, at least some bacteria do not secrete proteolytic enzymes. In the hydrolysis of lactose we have both dextrose and galactose which may in a great measure account for the retarded proteolysis of the proteins in the milk. The milk contains approximately 5 per cent lactose while the synthetic butter contains 1 per cent.

The other factor, the acidity of the media seems more evident in influencing the proteolytic changes. For the six organisms the average acidity of milk was 0.458 per cent at the end of forty-five days, at room temperature; that of synthetic butter 0.109 per cent at the end of sixty days at room temperature, and that of synthetic butter 0.07 per cent at the end of one hundred and twenty days at 0° to 4°C. The average per cent of acidity of the milk and synthetic butter at room temperature is approximately proportional to the concentration of the lactose. The high acidity in milk unquestionably influenced the normal development of the organisms as well as the activity of the enzymes.

The proteolytic enzymes formed by bacteria may be either tryptic or peptic or both, the action of the enzymes on proteins producing amino acids in addition to peptones, suggests the presence of tryptic enzymes. There is no evidence as yet to show that each group has an optimum activity at a certain degree of acidity or alkalinity, nor that all proteolytic organisms secrete enzymes of the same character.

In our study of B. panis migula (5), we found that when the acidity reached 0.45 per cent, both the production of acidity and proteolytic action ceased. The preliminary work suggests the possibility and fruitfulness of a more extended investigation of specific organisms and their enzymes. The influence of the

hydrogen-ion concentration on bacterial enzymes, the presence or absence of carbohydrates have not as yet been studied to warrant an *a priori* prediction.

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VARIATIONS IN THE SUSCEPTIBILITY OF THE FAT IN DRY WHOLE MILKS TO OXIDATION WHEN STORED AT VARIOUS TEMPERATURES AND IN VARIOUS ATMOSPHERES*

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Practical experience has shown that there is no great improvement in keeping quality of stored butters unless temperatures below 0°C. are used. Relatively low temperatures are therefore employed even when the period of storage is short.

The idea seems prevalent that in the case of dry whole milks a low temperature of storage is not an important factor. The work of Dahle and Palmer (1) is the only one reported wherein the effect of temperatures upon the rate of deterioration in dry whole milks has been noted. Their results are qualitative in nature only. They state that "the temperature at which the powders are stored proved to be an important factor. Not a great deal of difference was observed between powders stored at 4° and 20°C., but a great difference was observed when powders were stored at 37°C."

EXPERIMENTAL

A commercial sample of dry whole-milk powder was divided into two parts. One part of 1.54 per cent moisture content was divided into six samples, and each was stored at various temperatures ranging from 3° to 30°C. The other half of the commercial sample was divided into small lots and adjusted to higher moisture contents. The final moisture contents ranged from 2.60 to 3.40 per cent. Six samples from this lot were stored at the same temperatures as the samples of lower moisture content. The susceptibility to oxidation or the induction period of each

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sample was determined from time to time over a period of six months. (Lower induction period indicates increased susceptibility to oxidation.) The results of these experiments are shown in figures 1 and 2.

The first noticeable difference in the results with low and relatively high moisture content powders is that in the samples of low moisture content the increased susceptibility is greatest in the first sixty days of storage, while in those of higher moisture

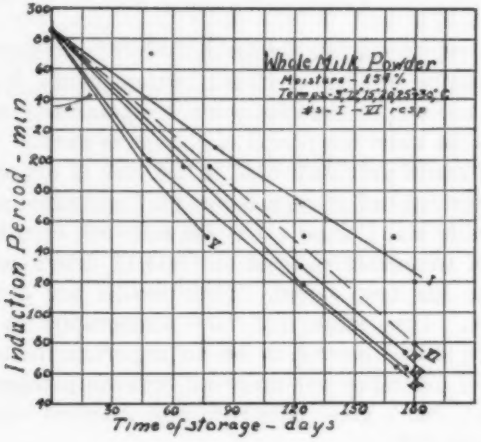

FIG. 1. SHOWING THE INCREASE IN SUSCEPTIBILITY OF THE FAT IN MILK POWDER OF LOW MOISTURE CONTENT TO OXIDATION DURING STORAGE AT VARIOUS TEMPERATURES

content the rate of deterioration is retarded during the first sixty days, but thereafter it is more rapid for the higher temperatures. The rate is more regular at lower temperatures. There is an indication that low temperatures retard the reaction fundamentally concerned in the oxidation when free moisture is present. Though the results shown in figure 2 are too inconsistent within themselves to warrant specific conclusions or calculations as to the effect of different moisture contents, a general comparison of the results with high and low moisture contents confirms our former observations that free moisture retards the rate of de-

terioration (2). This point is discussed later. In each case, for some unknown reason, results with sample 6 differ from those of the other samples, and this sample is therefore not considered representative of each lot.

Though the moisture content of the samples used in obtaining the values shown in figure 1 are not considered optimum for the best keeping quality of this milk, a further study of these values gives an idea of the temperature effects upon the rate of the

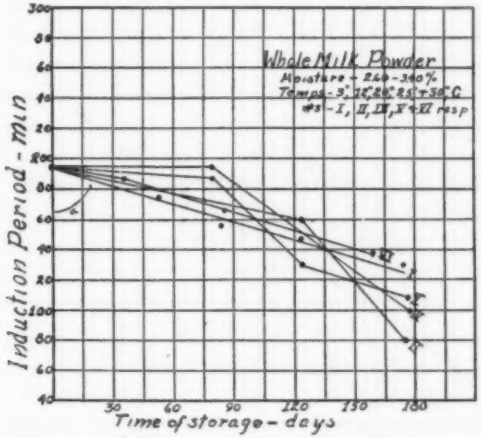

FIG. 2. SHOWING THE INCREASE IN SUSCEPTIBILITY OF THE FAT IN MILK POWDER OF RELATIVELY HIGH MOISTURE CONTENT TO OXIDATION DURING STORAGE AT VARIOUS TEMPERATURES

oxidation reaction where it is not complicated by the presence of any appreciable amount of free moisture.

The rates of increased susceptibility to oxidation (lowered induction periods) shown in figure 1 are measured by the angles represented by α, which the line for each sample forms with the ordinate. The greater the angle the less rapid is the rate of increased susceptibilities, and vice versa. Thus for sample 1 stored at 3°C. the rate is considerably less (greater angle α) than it is for sample 5 at 25°C. (smaller angle α). Numerical values for the rates of susceptibility changes are therefore ob-

tained by ascertaining the tangents of each angle. When these values are plotted against the temperatures of storage the relative value of each temperature over the higher temperature is noted.

Figure 3 indicates a regular increased keeping quality for each 5°C. decrease in the storage temperature between 25° and 10°C.

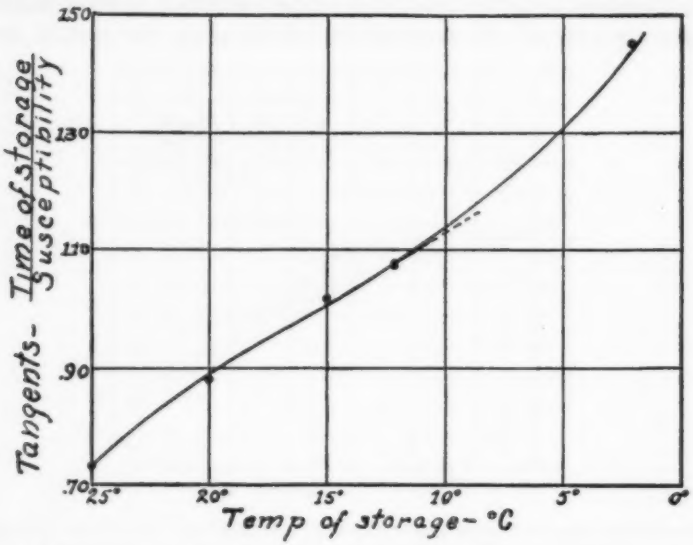

FIG. 3. SHOWING THE CHANGES IN TIME-SUSCEPTIBILITY RELATIONSHIP WITH TEMPERATURE OF STORAGE

Below 10°C. a marked increase in keeping quality is shown for each 5°C. decrease in storage temperature.

It may be stated that the dry whole milk of exceedingly low moisture content stored at 3°C. increased in susceptibility at approximately one-half the rate of the sample stored at 25°C. (tangents 1.45 and 0.72 respectively). This is also graphically shown in figure 1. A drop in the induction periods to any chosen value requires approximately twice as long a storage time at 3°C. as it does at 25°C.

With regard to dry whole milks of higher moisture content, another factor, namely, free moisture, is being dealt with. This factor affects the rate of the oxidation. As stated heretofore, the results shown in figure 2 are too inconsistent to warrant quantitative treatment. This figure, however, indicates that the rates are considerably less than those in figure 1, since the slopes of curves are greater with respect to the ordinate. Sample 1 in figure 2 shows a uniform rate of increased susceptibility. The tangent of the angle that this line forms with the ordinate is 4, while that of sample 1 in figure 1 is 1.4. This indicates that at 3°C. the rate of deterioration of a dry whole milk of exceedingly low moisture content is roughly 3 times as rapid as it is in a milk of higher vapor pressure where free moisture is present. The average rate of increased susceptibility for nos. 4 and 5 after eighty-five days storage is approximately equal to the rate of sample 1 (stored at 3°C.) and of figure 1, as expressed by the values of their slopes, 1.44 and 1.40 respectively. These figures indicate that moisture is a very critical factor in the study of keeping quality, especially in the region of 1.5 and 2.5 per cent.

These results confirm conclusions which the authors have reached in a previous work—that the optimum moisture content for spray powders of good solubility with respect to keeping quality is between 2 and 3 per cent. For powders with reduced solubility the optimum moisture content for increased keeping quality would be somewhat less, the vapor pressures being the same.

The subject of storage of products containing fats and oils, in vacuum or in inert gases, is one of considerable interest to the industry. Previous experiments with materials stored in carbon dioxide and in vacuum had given indefinite results. With a more accurate method available for measuring the changes that underlie deterioration and with a better knowledge of factors to be controlled, attempts again were made to determine quantitatively the rates of changes in susceptibility to oxidation during a storage in air, in partial vacuum, and in carbon dioxide.

Samples of the same dry whole milks of low and high moisture content were used in this experiment as were used in our previous

experiments upon temperature of storage. All samples were stored at temperatures ranging from 21° to 23°C. Samples were opened from time to time and the susceptibility to oxidation (induction period) was determined. The time when the first off odor was perceptible was also noted and is designated with an × in figure 4. An increase in the intensity of off odor is designated by an increase in the size of the character.

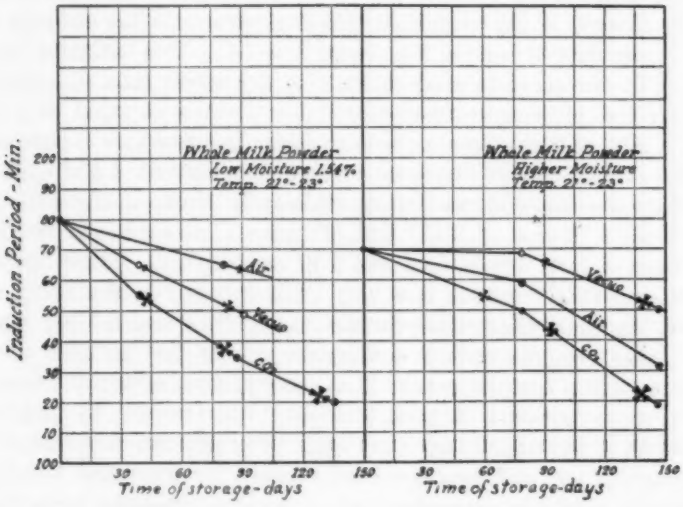

FIG. 4. SHOWING THE EFFECT OF VARIOUS ATMOSPHERES UPON THE SUSCEPTIBILITY OF THE FAT IN MILK POWDER TO OXIDATION DURING STORAGE

No great difference was noted between storage in partial vacuum and in air. Figure 4 indicates that for high and low moisture their relative values are reversed. In each case, however, storage in carbon dioxide proved the least efficient, the rate of susceptibility increase (lower induction period) being greater than for the other conditions shown. When judged by the olfactory sense dry whole milk stored in carbon dioxide always produced off odors and flavors before they were detectable in the samples stored in air or in partial vacuum.

These results are in accordance with what might be expected when the question is considered in the light of the effect of carbon dioxide upon soaps. It has been shown (3) that carbon dioxide can affect the splitting or hydrolysis of soaps (sodium salts of the fatty acids) and it seemed probable that the glycerides (glycerine + fatty acids) might also be affected in the same manner. This would produce free acids which are catalysts for the autoxidation of fats. These observations upon dry whole milks have been confirmed by results obtained upon pure milk fat.

DISCUSSION

The relative values for the susceptibility of fats to oxidation when stored at various temperatures, as shown in figure 1, were obtained upon dry whole milk which had not received clarifying treatment. Data previously presented indicated that this treatment of a milk materially improved the keeping quality of its dry whole milk. What effect this process may have upon the reaction involved is not known. The initial change that occurs in oxidation is undoubtedly modified greatly by the enzymes present, which are to a great extent removed in clarification. The reaction dealt with in the clarified product is therefore probably one of a different order and affected to a different degree by temperature changes. Further work will determine the variations of changes in the two products at different temperatures.

Results with dry whole milks of a low and relatively high moisture content indicate that free moisture has a decidedly retarding effect upon susceptibility to oxidation. For optimum keeping quality, therefore, a slight amount of free moisture is necessary. Since no two powders manufactured by different processes are alike with respect to their water adsorption capacities, each powder would have its own optimum.

For spray powders of good solubility the optimum moisture content seems to be between 2 and 3 per cent. The upper limit which can be used will be determined by several factors, namely, at what moisture content hydrolysis is promoted, and at what moisture contents solubility is affected by storage.

The data upon storage in various atmospheres are too meager to furnish any quantitative relationships. Repeated experiments with butteroil have confirmed the observations recorded. The results indicate that carbon dioxide can not be classed as an inert gas when its effect upon fats and oils are dealt with.

The results obtained with the samples stored in vacuum confirm previous results obtained in this laboratory. They indicate that keeping quality is largely dependent upon the inherent quality of the fat. The nature of the condition producing this inherent keeping quality has been shown to be due to some type of loosely bound oxygen which can not be removed by the use of vacuum. The amount of oxygen present in this form is usually sufficient to cause perceptible odors and flavors when it oxidizes the fat. Experiments with steam treated milk fat, reported in a former publication (4), tend to show this fact. Later experiments of more direct bearing upon this question are being published elsewhere (5).

Clarification seems to remove the catalysts necessary to the formation of these compounds and therefore improves the inherent quality of the fat. The effect of vacuum storage of a clarified product would probably therefore not be comparable to the results reported here.

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A METHOD OF INTERPRETING THE SCORES OF JUDGES OF DAIRY PRODUCTS*

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When competent judges are scoring cheese which have been made for experimental purposes, they are usually urged to place their scores upon each cheese without reference to the scores which other judges place upon the same cheese. Under such circumstances the judges will rarely score the cheese on exactly the same scale. The differences which result may be even greater than those which will be considered in this discussion. The determination of the significance of such scores is difficult and the problem is not limited to the scoring of cheese. It is encountered whenever an attempt is made to interpret the opinions of judges of milk, butter or ice cream when their judgments are recorded as numerical values upon more or less variable scales.

That the scores of the same cheese by different judges do not show exact agreement is illustrated in table 1, in which is presented a summary of the scores which three qualified judges placed upon a lot of 117 different cheese. The judges scored the cheese independently except when occasional comments were made upon exceptional samples.

Table 1 indicates rather strikingly that these judges agree upon neither the average score of the cheese nor upon the amount of variation in the scores of the series.

When the individual scores of each judge were examined, it was found that they were not using the same scale of points in judging the cheese. This is indicated in table 2 where the extreme variations of the judges' scores are shown.

From the combined evidence of tables 1 and 2, it is apparent that the mental standards of the judges in scoring cheese do not coincide. It does not follow that these judges are unable to

* Received for publication June 1, 1926.

select the best cheese and the poorest cheese and to place in their proper relative positions the cheese of all the intermediate grades. It is apparent, however, that the quality of a cheese scored 86 by A is not the equivalent in quality to the cheese which either B or C would designate by the same score. Judge A uses 17.75 points of score to indicate the same range of quality which B scores within a range of 12.50 points and C within 12.75 points. A point of score on the scale of points used by each of these judges should have the same significance to be comparable.

TABLE 1

The mean scores of 117 cheese estimated by three judges

JUDGE	MEAN SCORE	STANDARD DEVIATION	COEFFICIENT OF VARIATION
A	89.26 ± 0.19	3.03 ± 0.13	3.39
B	93.25 ± 0.18	2.91 ± 0.13	3.12
C	89.95 ± 0.16	2.58 ± 0.11	2.87

TABLE 2

The range of scores used by the three judges

JUDGE	SCORE OF THE BEST CHEESE	SCORE OF THE POOREST CHEESE	DIFFERENCE IN SCORE BETWEEN THE BEST AND POOREST
A	95.75	78.00	17.75
B	97.75	85.25	12.50
C	95.25	82.50	12.75

The fact that these judges examined the same lot of cheese must be emphasized. Since the range of score of each judge indicates the difference in quality between the best and the worst cheese, it seems logical to assume that the range of score of each judge should be numerically equivalent. These ranges of scores can be made numerically equivalent by assuming that the range in quality exhibited by the cheese of the series is equal to 100 points on a new scale in which the poorest cheese scores 0 and the best cheese scores 100. On the basis of this assumption it can be stated that A's range of score, which is 17.75 points, will equal 100 points on the new scale. B's range of score and C's range of

score of 12.50 points and 12.75 points respectively, will also equal 100. The score of any cheese of any judge can now be transposed to this common scale of points by a simple calculation which as a formula can be stated as:

$$x = \frac{100\ (s - m)}{r}$$

In this formula x equals the score of the cheese on the scale of 100; r equals the maximum actual score of the judge minus his minimum actual score; s equals the actual score of the cheese; and m equals the minimum actual score of the judge.

TABLE 3

The actual and ratio scores of 10 of the 117 cheese examined by the three judges

CHEESE NUMBER	JUDGE A		JUDGE B		JUDGE C	
	Actual score	Ratio score	Actual score	Ratio score	Actual score	Ratio score
1	91.50	76.0	92.50	58.0	89.25	52.9
2	88.00	56.3	94.25	72.0	91.50	70.6
3	88.50	59.1	92.50	58.0	92.00	74.5
4	90.50	70.4	96.00	86.0	91.00	66.6
5	88.50	59.1	95.25	80.0	89.50	54.9
6	95.75	100.0	97.25	96.0	95.25	100.0
7	90.75	71.8	94.50	74.0	91.00	66.6
8	94.25	91.5	95.75	84.0	95.00	98.0
9	95.50	98.6	96.75	92.0	95.00	98.0
10	93.25	85.9	96.25	88.0	93.50	86.3
Average.....	91.65	76.9	95.10	78.8	92.30	76.8

This "ratio score," as it might be called, indicates the quality of a cheese relative to the quality of all the other cheese in the lot examined.

To illustrate the application of this formula, the actual scores of judges A, B, and C on ten cheese selected at random from the 117 examined are presented in table 3 with the equivalent ratio scores calculated from the value of r and m given in table 2. Judge A, for example, gives cheese no. 1 in table 3 an actual score of 91.50. His maximum actual score, 95.75, less his minimum

actual score, 78.00, gives a value for r of 17.75. Substituting in the formula:

$$x = \frac{100\ (91.50 - 78.00)}{17.75} = 76.00$$

This ratio score method of interpreting the significance of the scores of the cheese judges is not advanced with the idea that it is a perfect solution of the problem but rather as a tentative approach toward a common method of showing the results of experimental work where the quality of the product is involved. The method may be criticized adversely because it depends on the extreme range of the actual scores. Judges may allow their liking for a good product to influence unduly their highest score and may also cut too severely the score of the poorest sample. On the other hand this treatment of the judges' scores gives a series of numerical values which indicate the quality of the cheese examined on a scale of points common to all the judges. These values may be more correctly used than the judges' actual scores in determining the mean, standard deviation or the probable error of the series.

A PHOTOGRAPHIC METHOD FOR OBTAINING ACCURATE MEASUREMENTS OF ANIMALS*

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In connection with certain feeding experiments under the direction of Prof. H. B. Ellenberger,[1] it seemed desirable to obtain photographic records of the size and condition of the animals at frequent intervals. In pictures of this sort which have been made hitherto, the measurements usually have been indicated by a background, placed behind the animal, and ruled off in squares, or by a screen of intersecting wires placed in front of the animal. Both of these methods are open to criticism since they do not give a true measurement. The ruled background makes the animal appear larger than it actually is, while the screen in front reduces the apparent size.

This condition is shown diagrammatically in figure 1. The line CC represents most nearly the true measurement of the animal, the shorter line AB the size indicated by the screen, placed in front, and the longer line $A'B'$ the size as shown on the background.

In order to avoid the error involved by the use of either of the two methods just described the writer worked out a method which records the actual size of the animal. Bars, scaled off at the desired intervals, were arranged to make a frame entirely surrounding the subject so that the animal could be placed in such a manner that the plane of the center of its body corresponded with that of the frame which gave the scale of measurement. An ordinary background was used and the frame was so made that it could be moved forward or backward and set at any desired distance away from the background. The accompanying pictures show the details of the construction of this apparatus.

* Received for publication January 2, 1926.

[1] Head, Department of Animal and Dairy Husbandry, University of Vermont and Vermont Experiment Station.

Figure 2 shows the frame surrounding the animal. The section of the frame resting on the platform upon which the animal stands is also shown in figure 5. The white marks on this section of the frame in this picture indicate the scale of measurement corresponding to that on the sides and top of the frame as shown in figures 2 and 4. Figure 3 shows the relative position of the frame and the animal. The bottom supports of the frame slide backward or forward in grooves and the position of the frame may be

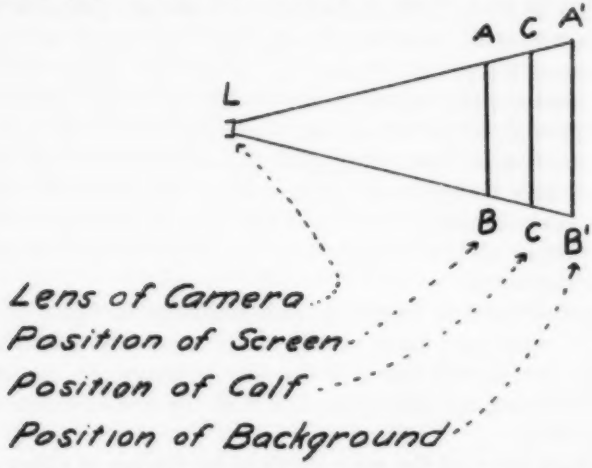

FIG. 1. THE OBJECT TO BE MEASURED MUST BE IN THE SAME PLANE AS THE SCALE OF MEASUREMENT

readily changed and fastened securely by means of ordinary clamps.

In order to have the camera at the desired place each time, an adjustable camera stand was built so that the camera could be set at a known height above the platform. A track was built in front of the platform running at exactly right angles to it. The platform and track were leveled up so that the camera would be perfectly square with the background in whatever position the camera was placed. The slide on the camera stand, and also the track were marked with a scale so that any position of the camera might be recorded and duplicated if desired.

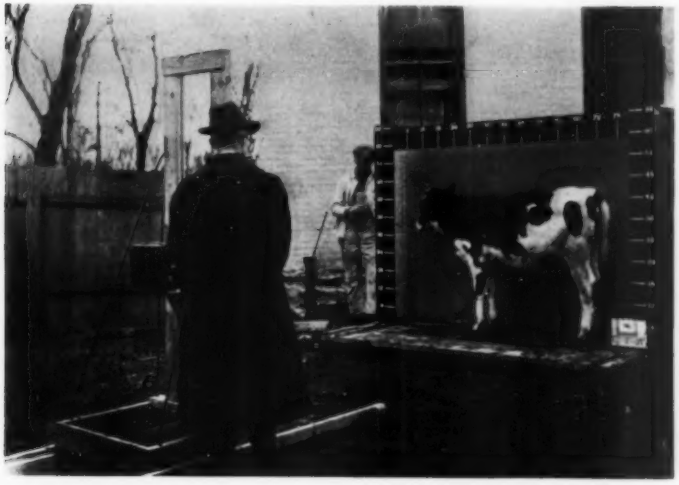

FIG. 2. THE ARRANGEMENT OF THE CAMERA, FRAME AND BACKGROUND

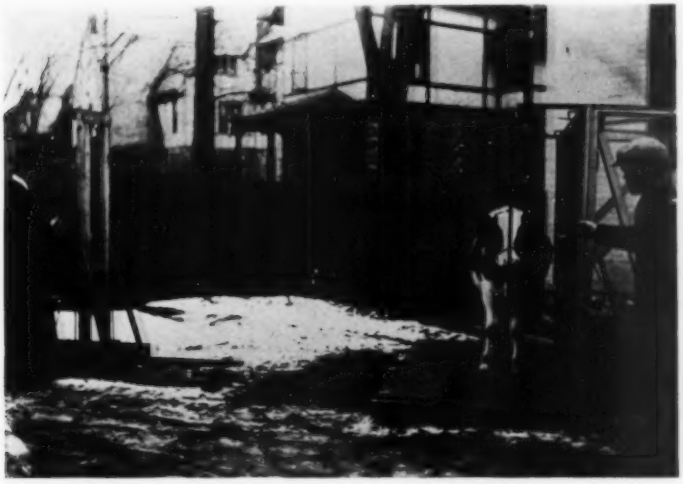

FIG. 3. THE POSITION OF THE ANIMAL IN RELATION TO THE FRAME

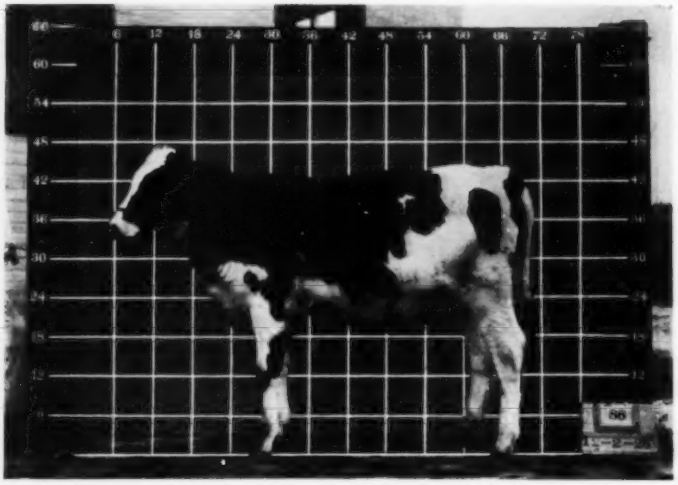

FIG. 4. MEASUREMENTS BY THE IMPROVED METHOD

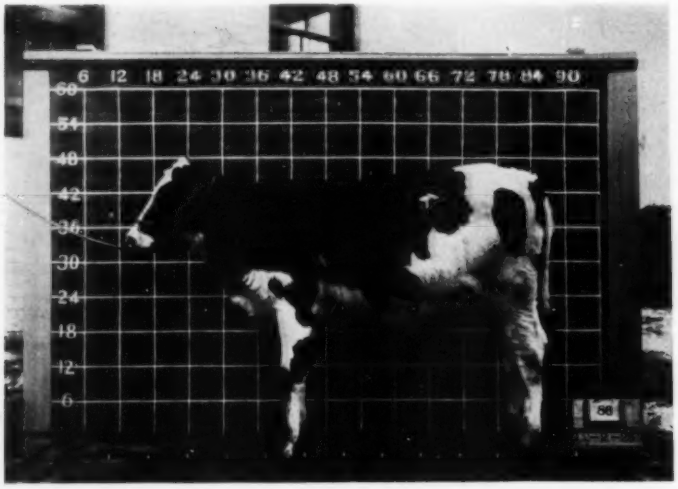

FIG. 5. MEASUREMENTS BY THE RULED BACKGROUND METHOD

After the negative is made the squares are ruled off with a ruling pen, using waterproof black India ink, and stopping when the line strikes any portion of the animal's body. Thus when the prints are made the lines show up white. In the case in question, as the calves were black and white, a gray background was used which would contrast with both the black and the white. Of course if it is desired, the lines can be ruled on the finished print.

The difference in the size of a six months' old calf photographied against a ruled background and by the improved method is shown in figures 4 and 5. Figure 4 illustrates the improved method and figure 5 the ruled background. The camera was exactly the same distance from the calf in each case but the size of the calf as indicated by the scale is quite different.

Although this method is not quite as simple as those that have been previously used, yet it would seem that the increased accuracy obtained is worth much more than the extra work involved.

STUDIES ON YEASTS IN DAIRY PRODUCTS

II. GENERAL GROUPING OF THE MORE NUMEROUS TYPES

W. A. CORDES[1] AND B. W. HAMMER

One of the objects of the studies that have been carried out by the dairy section of the Iowa Agricultural Experiment Station on the yeasts found in dairy products has been the eventual development of a system of classificaton that will make possible the easy and accurate identification of yeast cultures isolated. Because of the wide range of forms found among the yeasts such a classification necessitates a separation of the organisms into groups with those in each group having relationships, more or less close, that naturally throw them together. A grouping that has been in use for several years is herein presented with the idea that it may serve as a starting point in the development of a scheme of classification for the yeasts found in dairy products; a rearrangement and further division of the groups is to be expected but can only be made as a result of a detailed study of the organisms belonging to each.

The organisms in certain of the groups have been studied in considerable detail while those in other groups have not. The results obtained indicate that the grouping adopted, although incomplete, is of help in a consideration of the yeasts isolated from milk and cream and their derivatives.

Grouping of yeasts found in dairy products

A. Group 1. Yeasts producing conspicuously colored colonies.
  a. Producing pink colonies.
    Includes *Torula glutinis* and also other forms.
  2a. Not producing pink colonies.
    b. Having yellow colonies due to associative action with *Aspergillus niger*.
      Various species of yeasts are here involved.
2A. Yeasts not producing conspicuously colored colonies.
  B. Group II. Yeasts producing dull, spreading, irregular-edged colonies on whey agar.

[1] Formerly assistant in dairying, Iowa Agricultural Experiment Station.

a. Growth very thin on whey agar; action on sweet milk inconspicuous.
Includes *Mycoderma monosa*.

2a. Growth on whey agar flat but not thin as under "a"; rapid digestion of sweet milk.
Includes a type commonly found but not yet described.

2B. Yeasts not producing dull, spreading, irregular-edged colonies on whey agar.

C. Group III. Yeasts showing white, smooth-edged colonies and pronounced gas formation in milk.

a. Optimum near 37°C.; cells oval. *Torula cremoris*.

2a. Growth slow if at all at 37°C., but good at 30°C.; cells spherical. *Torula sphaerica*.

2C. Group IV. Yeasts showing white, smoothed-edged colonies but *no* gas formation in milk—common white yeasts.

a. Gas produced is saturated sucrose bouillon.
Includes *Torula lactis-condensi*, and probably other forms.

2a. No gas in saturated sucrose bouillon.
Includes a great variety of species many of which produce no change in sweet milk.

Groups I and II are separated from the other groups on the basis of the appearance of the colonies; this is done primarily for the reason that the first information in regard to a culture isolated relates to its colony appearance and accordingly any division that can be made on such a basis is very desirable. The division of the organisms not included in groups I and II cannot be made on the colony basis because there is nothing characteristic, but the organisms belonging to group III can easily be separated from those of group IV by inoculating into litmus milk and examining for gas formation; after some little experience the organisms of group III can be recognized by the odor produced in pure cultures in agar, milk, or other materials. Group IV is really a catchall group which further studies are certain to divide up; the organisms in this group apparently present the greatest difficulties from the standpoint of an adequate classification.

The groups established in the scheme are believed to be arranged in such a way that the one to which a given organism belongs can be determined in the easiest possible manner. The group of colored colony yeasts is put first because one of the most striking and easily noticed characters of a colony is its color; in young colonies the color production may not be as pronounced as in older ones, but any grouping involving cultural characters must necessarily be dependent on cultures that had been allowed

a reasonable time to develop. In subsurface colonies the color of the pink yeasts is often not as pronounced as in surface colonies but ordinarily it is intense enough to be easily recognized in plates that are several days old. The dull, spreading, irregular-edged colony distinguishing group II is another striking character that is quite easily recognized and readily distinguished from the smooth-edged, shiny colonies found with the remaining groups. Groups III and IV cannot be separated on the colony basis but group III is characterized by lactose fermentation and this is easily determined by observing the changes occurring in inoculated milk. The odor produced during the lactose fermentation is quite characteristic so that the organisms belonging to group III can often be selected on this basis also.

Group IV, the common white yeasts, is the group which experience suggests will be certain to require further division. There are wide variations in the yeasts here included but the work so far done does not suggest any very logical basis for division. Distinct species can easily be separated but any relationship between these is not as yet clearly evident. The formation of gas from strong sucrose solutions, e.g., saturated sucrose bouillon, is a suggested basis for the first division, mainly because the yeasts effecting this change are so important in the sweetened condensed milk industry; however, there seem to be organisms that can ferment saturated sucrose bouillon that cannot ferment sweetened condensed milk.

The grouping given does not include all the yeasts that can be isolated from dairy products, but does include those that have been isolated often enough to be considered of significance. For example, a yeast producing a brown color was isolated from cream and there is no provision for this in the scheme of grouping, although a place could easily be provided. This yeast, however, has never been found again and while it is of interest because of the rarity of yeasts producing a brown color, a study of only one culture cannot give an adequate idea of it so that its description had best be left until other cultures are found or some special importance seems attributable to it. Descriptions of organisms based on only one culture cannot take into account the variations occurring in the species and are thus likely to lead to needless confusion in the literature.

THE USE OF VARIOUS FORMS OF OXYGEN IN THE TREATMENT OF ABNORMAL FERMENTATION IN SWISS CHEESE*

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One of the most common difficulties encountered in the manufacture of Swiss cheese is the development of the so-called nissler fermentation. A nissler fermentation is one in which the gas-producing organisms are present in such numbers or in such a state that the ordinary lacto bacilli in the whey rennet or "Lab," or the bulgaricus starter fail to hold the gas-producing organisms in check, and as a result the cheese does not have a firm, solid curd but is filled with numerous small holes sometimes one-sixteenth to one-eighth of an inch in diameter. Such a cheese is spoken of as a nissler, or a cheese with a thousand eyes. When a very vigorous and rapid fermentation takes place while the cheese is on the press so that the cheese huffs and may spread over the side of the press hoop, it is spoken of as a pressler cheese. Either of these fermentations may result from overripe or dirty milk, from contamination through unclean utensils, or even from the use of overripe or gassy "Lab" or "sour." The prevalence of these abnormal fermentations follows the seasonal temperatures closely. In general, the highest percentage occurs during the summer months; the lowest in the spring, fall, and winter months.

At Grove City, Pa., where milk is delivered once a day, resulting formerly in a high percentage of nissler cheese during the summer months, it has been found that during this period the number of such cheeses could be reduced to a comparatively low percentage by chilling the milk to 50°F. as soon as it reaches the creamery. In the case of milk delivered once a day, the nissler fermentation often manifests itself while the cheese is

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on the press and is ordinarily determined by sounding with a large spoon. In this case no huffing is in evidence. If a cheese sounds more or less over the entire surface instead of in one or two spots, it will very likely prove to be a nissler. Occasionally a cheese will sound over the entire surface and still not show any indications of gas except within an inch or so of the edge. Such a condition may be brought about as a result of too rapid cooling or of too frequent turning while on the press. When a cheese sounds in only one or two spots it is probably an indication of whey pockets which, as the name suggests, are merely isolated areas where whey collects and is not properly expelled.

Another means of determining whether or not a cheese is a nissler is by examining it with a small Roquefort trier at the time it is removed from the brine tank. If no holes are present in cheese made from milk delivered once a day, it probably is not a nissler, although this fermentation may occasionally develop later in the curing process.

In case of abnormal fermentations in cheese made from milk delivered twice a day, the cheese seldom sounds while on the press; and very careful examination is often required later to detect the minute holes which, when present, are a fairly certain indication of a nissler condition. Some manufacturers claim that these pin holes do not develop for several days after the cheese has been removed from the brine tank.

Pasteurization has been used to a limited extent in an effort to control these types of fermentation. A patented process, however, prevents the general use of the holding system of pasteurization of milk for the manufacture of Swiss cheese. Results obtained by the holding system of pasteurization have not always proved satisfactory as the cheese manufactured after the milk has been pasteurized often cracks or checks internally, and at times an abnormal fermentation may even develop causing it to be no better than a No. 2 grade. In general, pasteurization of milk for making this kind of cheese tends to delay the eye formation until the curd has lost some of its normal elasticity; and as a result, the cheese either checks or has a tendency toward becoming glaesler. A glaesler cheese is one that usually cracks or splits

crosswise to the flat surface, or one near the surface of which small checks form which frequently are only an inch or so in length. Unless the cheese is made on an extensive scale the cost of equipment and the extra labor required makes the pasteurization of milk for Swiss cheese impractical for the average factory.

In factories receiving milk once a day it appears that it is the anaerobic rather than the coli-aerogenes types of organisms which are primarily responsible for these abnormal fermentations.

At present the most practical means of controlling these abnormal fermentations is by the production of clean, quickly cooled milk and the use of an active, pure bulgaricus culture. Even when these conditions prevail, cheeses of either the nissler or pressler type sometimes result.

Since bacteriological studies have often indicated the presence of anaerobic spore-forming bacteria in the milk and cheese, the use of some form of oxygen as the means of treating milk suggests itself. By adding gas-producing anaerobic cultures to milk, studies have been made on the effect of the use of ozone, oxygen and aeration in checking subsequent gassy fermentations in Swiss cheese.

THE USE OF OZONE IN THE TREATMENT OF MILK

Attention was first directed toward the possibility of utilizing gas in the treatment of abnormal fermentation occurring in milk as a result of an article on this subject by Emil Weiner and W. Freund (Vergl. W. Freund, Chem. Ztg., 1911, S. 905; and Weiner klin. Wochenschr., 1910, Nr. 26). A summary of Weiner's results is as follows: Very good results were obtained in the sterilization of milk by first atomizing the milk and then blowing through it a very strong concentration of ozonized air (0.03 to 0.4 gram per cubic meter) and following this by reatomizing the milk and blowing it thoroughly with sterile air so as to drive off the surplus ozone. In this way it is claimed that the treated milk was absolutely freed from all pathogenic bacteria. It appears that the acid bacteria of milk were not much affected but that the ozone was extremely active toward foreign organisms. Further claims are made that when milk is treated in this way, the taste

and odor of the milk are not affected, nor are the vitamins or proteins. Doctor Freund also used ozone in his attempts to sterilize milk by running ozonized air through milk for a period of ten minutes. This treatment rendered the taste and odor of the milk so offensive that it could not be used for practical purposes. In order to use ozone it is necessary to atomize the milk so as to expose a maximum surface. It is also essential to dispose immediately of the surplus ozone by reatomizing the milk and blowing it with sterilized air.

The practicability of atomizing milk in order that it might be more effectively treated with ozone and further treated with sterile air did not seem to offer any possibilities, at least so far as the manufacture of Swiss cheese is concerned. There seemed a possibility that the injurious effects of the ozone might be dissipated during the process of manufacture and curing of cheese.

METHOD OF TREATMENT

All milk used in these experiments was first mixed in a large holding tank, weighed, and equal quantities run into small experimental kettles, each holding approximately 700 pounds. The milk was thoroughly mixed with the exception of a small portion used for standardizing purposes. This milk was put into a kettle and heated to the proper temperature, separated, and then equal portions added to the experimental kettles. Each kettle received similar quantities of the Bacillus bulgaricus, the eye culture, and rennet in suitable proportions. In addition to the usual Swiss starter a gas-producing spore-forming anaerobe which was originally isolated from a Swiss cheese was also added. The purpose of this organism was to develop a gassy fermentation in the cheese. Only one kettle was treated with oxygen, the other served as a check. Each pair of cheeses was manufactured as nearly alike as possible. The quantity of milk used each day varied somewhat, but usually 450 to 650 pounds of milk were used for each cheese.

The ozone was prepared by running air first through sulphuric acid and then through an ozone machine in which currents of air passed between two highly charged electric plates. The ozone

thus generated was conveyed from the machine to the milk by means of a glass tube with connections sealed with paraffin. The ozone was not as a rule run through the milk vigorously but at a fairly uniform rate of possibly 15 to 20 cubic feet per half hour the milk being stirred meanwhile by means of an agitator. At the conclusion of the ozone treatment, air was blown through the milk rather vigorously for five to ten minutes. It was possible to record only the time at which the ozone was run in, or the number of cubic feet of the gas used, since the quantity of ozone actually absorbed by the milk could not be measured. As indicated in table 1 ozone was run into milk for periods varying from fourteen to forty-five minutes. Since the presence of ozone gas coming from the milk was easily discernible by its characteristic odor, there could be but little question as to when the gas was generated. With the exception of a few cases, the same quantity of milk was used in each kettle. In these cases the difference in the weight of the milk was only about 20 pounds so that this variation would not seem sufficiently great to influence appreciably the final results.

The ozone was run into the milk at temperatures varying from 3° to 33°C.; in a few cases no record was made of the temperature. When this was done the milk was not warmed and seldom ran above 12°C. In all cases the temperature of the check kettle was held as nearly like that of the treated kettle as possible. The anaerobic culture was added to the milk just before the introduction of the ozone gas, the bulgaricus and eye cultures just before the addition of the rennet.

DISCUSSION OF RESULTS

Table 1 gives the results obtained by adding an anaerobic culture to milk and treating it with ozone. About 44.44 per cent of the cheeses showed no gas, 38.88 per cent showed improvement while 16.66 per cent showed no improvement as compared to the check cheeses. Seventy-five per cent of the ozonated cheeses had an off flavor, while 25 per cent showed no off flavor.

In many cases the cheeses were huffed so much that the edges

TABLE 1

Effect of ozone on the flavor and gassy fermentations of Swiss cheese

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OZONE RUN	TEMPERATURE OF MILK WHEN TREATED	ACIDITY BULGARICUS CULTURE	GRADE OF CHEESE*	EFFECT OF OZONE†	PRESENCE OF OFF FLAVOR‡	REMARKS
	pounds	*cc.*	*minutes*	*°C.*	*per cent*				
1	600	1,350	30	Below 12	1.70	No. 1 g	+		Slight off flavor
1-1§	600	1,350	None		1.70	No. 1 f		Yes	Nissler outer edge
2	600		45	Below 12	1.52	No. 1 p	−	Yes	Slight off flavor
2-1§	600		None	Below 12	1.52	No. 2			
3	525	1,000	30	Below 12	0.76	No. 2	0	−	Pressler cut morning after making
3-1§	525	1,000	None	Below 12	Whey	No. 2			Pressler cut morning after making
4	650	1,000	14	30		No. 2	0		Nissler edge. Possibly too little ozone
4-1§	650	1,000	None	30		No. 2		−	Nissler edge
5	575	800	30	30	1.53	F. f	+		No off flavor
5-1§	575	800	None	30	1.53	No. 1 p		No	Nissler at edge
6	540	1,300	45	30	1.52	F. f	−		Not nissler. No off flavor
6-1§	540	1,300	None	30	1.52	No. 1 g		No	Not nissler. No off flavor
7	510	800	45	15	1.30	No. 1 f	+		Flavor hopelessly off
7-1§	510	800	None			No. 1 g		Yes	Trace of nissler at edge
8	540	1,250	30	Below 12	1.60	F. p	+		Flat poor flavor not nissler
8-1§	540	1,250	None		1.60	No. 2		No	Nissler edge

9	530	925	45	15	1.30	No. 1 p	X		Very much off flavor. Little nissler at ends
9-1§	530	925	None	15	1.30	No. 2		Yes	Nissler 2 inches at ends
10	550	950	45	15	1.40	No. 2	X		Trace of nissler, cut morning after making
10-1§	550	950	None	15	1.40	No. 2		–	Slightly gassy throughout
11	650	1,225	30	25	1.36	No. 1 f	+		Ozone flavor plainly noticeable. Not nissler
11-1§	650	1,225	30	25	1.36	No. 2		Yes	Nissler 2 or 3 inches at edge
12	600	1,000	30	30	1.65	No. 1 g	X	No	Trace of nissler. Very little if any off flavor
12-1§	600	1,000	30	30	1.65	No. 1 f			Somewhat nissler at edge
13	525	800	30	25	1.32	No. 1 f	+		Distinct ozone flavor. Not nissler
13-1§	525	800	30	25	1.32	No. 1 f		Yes	Trace of nissler
14	530	850	45	Below 12	1.30	No. 2	X	–	Somewhat nissler. Cut morning after making
14-1§	530	850	45	Below 12	1.30	No. 2			Less nissler. Cut morning after making
15	625	950	23	33	1.72	No. 1 p	X		Somewhat nissler. Very much off flavor
15-1§	625	950	23	33	1.72	No. 2		Yes	Pressler. Cut morning after making
16	640	1,000	30	30	1.70	No. 1 f	+		Not nissler. Distinct off flavor
16-1§	657	1,000	30	30	1.70	No. 2		Yes	Nissler 1 or 2 inches at edge

TABLE 1—*Continued*

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OZONE RUN	TEMPERATURE OF MILK WHEN TREATED	ACIDITY BULGARICUS CULTURE	GRADE OF CHEESE*	EFFECT OF OZONE†	PRESENCE OF OFF FLAVOR‡	REMARKS
	pounds	*cc.*	*minutes*	*cc.*	*per cent*				
17	600	1,000	30	31	1.50	No. 2	0	–	Both cheese nissler. Bulgaricus added before ozone
17-1§	600	1,000	30	31	1.50	No. 2			
18	640	1,000	30	30	1.40	No. 1 f	+		Not nissler. Distinct off flavor
18-1§	640	1,000	30	30	1.40	No. 2		Yes	Somewhat nissler and very much over set
19	600	1,100	30	30	1.75	No. 2	X		Trace of nissler. Has ozone flavor
19-1§	600	1,100	30	30	1.75	No. 2		Yes	Pressler and nissler
20	676	1,000	30	30	2.01	No. 2	X		Trace of nissler. Distinct off flavor
20-1§	704	1,000	30	30	2.01	No. 2		Yes	More or less gassy throughout
21	580		30	30	1.48	No. 1 f	–		Distinct off flavor
21-1§	580		30	30	1.48	No. 1 g		Yes	

* F = fancy (Fancy and No. 1 further divided: g = good; f = fair; p = poor).

† + = completely checked; X = improvement; 0 = no improvement; – = no comparisons could be made.

‡ – = no comparisons could be made.

§ check cheese.

extended over the edge of the hoop and were so badly pressler that further experimentation could not be carried on, and they were accordingly cut for examination. In a few cases the cheeses showed nissler tendencies throughout, but more often the gas was only in evidence for a few inches at the edge, or at those points where the cheeses cooled the quickest. With large cheeses weighing over 100 pounds there is often a difference of from 2° to 3°C. between one point of the cheese and another. In a few days there was only a trace of gas in the interior, a condition which may occasionally happen whether or not gas cultures are added to the original milk. The cheese indicated by the positive sign in table 1 showed no gas either at the edge or at any point on the interior.

In cheese 3 a whey starter was used instead of the usual skim milk starter. In this particular case the acidity was somewhat higher than that usually employed with whey starters. A very high acidity in either whey or skim milk starter caused by too long an incubation period may weaken the organisms to such an extent that they fail to function normally.

In cheese 4 the ozone was run into the milk for fourteen minutes whereas in most cases it was run for about thirty minutes. This may have been an insufficient treatment of the milk.

In cheese 17 the bulgarian culture was added to the milk prior to the treatment with ozone instead of later, as was done in all other cases. It is possible that the ozone may have influenced the development of the bulgarian culture.

In those cases where an improvement was noted as a result of the ozone treatment, the check cheese showed gas to a greater distance from the surface, or to a greater degree.

There was a distinct off flavor in 75 per cent of the ozone-treated cheese. The flavor of the cheese was comparable to the characteristic odor of the gas. The intensity of the flavor in the ozonized milk cheese seemed to vary somewhat from day to day even when the milk was treated in the same manner. Generally, however, the defective flavor was so pronounced as to render the cheese unmarketable. Four of the ozonized milk cheeses showed no noticeable off flavor, but in most cases an unpleasant flavor,

TABLE 2

Effect of oxygen on the gassy fermentation in Swiss cheese

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OXYGEN RUN	QUANTITY OF OXYGEN	TEMPERATURE OF MILK WHEN TREATED	GRADE OF CHEESE*	EFFECT OF OXYGEN†	REMARKS
	pounds	*cc.*	*minutes*	*cubic feet*	*°C.*			
1	500	1,400	30		16	No. 2		Pressler
1-1†	500	1,400		None		No. 2	0	Pressler
2	419	850	20		16	F. p		Not nissler
2-1‡	419	850		None		F. f	+	Nissler at edge
3	460	1,050	20		15	No. 2		Not nissler
3-1‡	460	1,050		None		No. 2	+	Nissler at edge
4	460	1,025	20		9	No. 1 f		Not nissler
4-1‡	460	1,025		None		No. 1 f	+	Nissler at edge
5	360	725	15		9	No. 2		Pressler
5-1‡	360	725		None		No. 2	0	Pressler. No difference
6	369	1,000	20		8	F. f		Not nissler
6-1‡	369	1,000		None		No. 2	+	Pressler. Cut morning after making
7	369	950	15		8	No. 1 f		Nissler at edge
7-1‡	369	950		None		No. 2	X	Pressler
8	533	1,100	10		8	No. 1 f		Not nissler
8-1‡	510	1,100		None		No. 1 f	+	Trace of nissler
9	370	2,000	20		6	No. 1 f		Not nissler
9-1‡	370	2,000		None		No. 2	+	Pressler

10	370	1,000		10	6	No. 2		Nissler about 1 inch at edge
10-1‡	370	1,000		None		No. 2	X	Nissler throughout
11	370	1,000		20	7	No. 1 f		Not nissler
11-1‡	370	1,000		None		No. 1 g	+	Nissler at edge, 1 to 2 inches
12	370	1,000	—	10.5	7	No. 2		Pressler
12-1‡	370	1,000	—	10.5		No. 2	0	Pressler. No difference
13	370	1,000	—	11.2	8	No. 1 f	+	Not nissler
13-1‡	370	1,000		None		Cut		Pressler. Thrown out morning after making
14	370	1,000	—	11.2	8	No. 2	0	Nissler and pressler
14-1‡	370	1,000	—	None		No. 2		Nissler and pressler
15	370	1,000	—	17	8	F. p	+	Not nissler
15-1‡	370	1,000		None		Cut		Pressler. Cut morning after making
16	370	1,000	—	17	6	F. f	+	Not nissler
16-1‡	370	1,000		None		No. 1 p		Nissler at edge
17	370	1,000	—	17	6	F. p		Trace of nissler
17-1‡	370	1,000		None		No. 1 p	X	Nissler 2 or 3 inches from edge
18	370	1,000	—	18	10	F. p.	+	Not nissler
18-1‡	370	1,000		None		F. g		Nissler at edge
19	370	1,000	—	9.6	6	No. 2	0	Nissler at edge
19-1‡	370	1,000		None		No. 2		Nissler at edge
20	370	1,000	—	20.5	8	No. 2	X	Nissler at edge
20-1‡	370	1,000		None		No. 2		Nissler throughout

TABLE 2—*Continued*

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	LENGTH OF TIME OXYGEN RUN	QUANTITY OF OXYGEN	TEMPERATURE OF MILK WHEN TREATED	GRADE OF CHEESE*	EFFECT OF OXYGEN†	REMARKS
	pounds	*cc.*	*minutes*	*cubic feet*	*°C.*			
21	370	1,000	—	20	13	No. 2	X	Nissler at edge
21-1‡	370	1,000		None		No. 2		Nissler throughout
22	370	1,000	—	23	16	F. f	+	Not nissler
22-1‡	370	1,000	—	None		No. 2		Genuine nissler with abortive eyes
23	370	1,000	—	17.5	16	F. p	+	Nissler
23-1‡	370	1,000	—	None		No. 2		Perfect nissler with abortive eyes
24	370	1,000	—	10.2	16	F. f	+	Not nissler
24-1‡	370	1,000		None		No. 2		One-half cheese nissler, pressler at edge and top and for 1 to 2 inches
25	370	1,000	—	5.5	17	F. f	+	Not nissler
25-1‡	370	1,000		None		No. 2		Nissler at edge
26	370	1,000	—	20.5	17	No. 2	X	Slightly nissler, center nissler
26-1‡	370	1,000		None		No. 2		Pressler and showed large holes
27	370	1,000	—	30	11	No. 1 g	+	Not nissler
27-1‡	370	1,000		None		No. 2		Genuine nissler with abortive eyes

* F = fancy (Fancy and No. 1 further divided; g = good; f = fair; p = poor.)
† + = completely checked; X = improvement; 0 = no improvement.
‡ check cheese.

which failed to disappear during the curing process, developed the day after making.

While the use of ozone might prove an economical means of treating milk containing anaerobes, the fact that the resulting cheese in most cases showed a distinct off flavor makes it appear doubtful whether such a gas could ever be used commercially for the treatment of milk used in the manufacture of Swiss cheese.

THE USE OF OXYGEN IN THE TREATMENT OF THE GASSY FERMENTATION IN SWISS CHEESE

In conducting these experiments oxygen was run into milk through a glass tube to which a perforated coil was attached, thus allowing a better distribution of the gas. The temperature of the milk varied from 6° to 17°C., the qualtity of gas used from 5.5 to 30 cubic feet per kettle, and the cheeses were made experimentally as with the ozone treatment. Table 2 gives the results obtained by adding a pure culture of a spore-forming anaerobe to milk and treating it with oxygen.

DISCUSSION OF RESULTS

Somewhat better results were obtained from the use of about 20 cubic feet of oxygen instead of 10 cubic feet. However, as in the case of the ozone, oxygen did not always check the gassy fermentation. Negative results were obtained when oxygen was added to the milk at both a high and a low temperature.

The effect of oxygen in the treatment of milk to which pure cultures of gas-producing spore-forming anaerobes were added is summarized as follows:

In about 59.25 per cent of the cheeses made the gas was completely checked by oxygen, in 22.22 per cent there was an improvement, and in 18.51 per cent no improvement was noted as compared to the check cheeses.

Nine pairs of experimental cheeses were made on a factory scale. One half of them received oxygen, while the remainder were used as controls. The milk was first mixed in a holding vat, divided, and then manufactured into cheeses as nearly alike as

possible. Unfortunately from an experimental standpoint, only two cheeses showed nissler tendencies. With these two pairs of cheeses there was a striking contrast. In one case the oxygen-treated cheese was a good No. 1, in the other case a fancy, whereas the untreated cheeses were both No. 2. Neither of the No. 2 cheeses was, strictly speaking, pin-eyed nissler, but both were filled with numerous small eyes perhaps $\frac{1}{8}$ to $\frac{1}{4}$ inch in diameter. These cheeses were made from 1550 to 1650 pounds of milk; the oxygen-treated cheese received 100 cubic feet of the gas in the original milk. In the other seven pairs of cheeses there was but little difference between the treated and untreated cheeses. The results here seem sufficiently favorable to warrant further experimentation to determine just how small a quantity of oxygen might be used to give effective results.

During the initial stage of eye development there was a marked contrast in eye formation between the nine pairs of treated and untreated cheeses, but upon final examination this earlier improvement was less marked except in the two cases above mentioned. These cheeses were all cut and sold locally, thus affording a good opportunity for careful examination.

One advantage in the use of oxygen is the fact that it has little or no injurious effect upon the flavor of the cheese under the laboratory conditions of the Bureau of Dairy Industry. With quantities of oxygen used it would cost about $1.00 to treat the milk for 150 to 160 pound cheeses. It is possible that in the experiments carried on in the laboratory when gas-producing cultures were added their number would be considerably in excess of what would normally be found in commercial milk. The gas cultures were grown in skim milk and thus particles of curd were introduced into the milk, a condition which does not afford so favorable an opportunity for oxygen treatment as in the case of commercial milk.

THE GERMICIDAL AND INHIBITORY ACTION OF OXYGEN

The action of oxygen on a spore-forming anaerobe used in these experiments indicates that the gas has a germicidal and

inhibitory action on the organisms as indicated by dilution tests. Flasks containing 1000 cc. of milk were sterilized, cooled and inoculated with 2.5 per cent of the freshly grown gas-producing culture. Oxygen was run into half the flasks for periods of five, fifteen, and thirty minutes, while the remainder of the flasks served as checks. Dilution tests were then made in freshly sterilized milk tubes up to the sixth dilution from both the treated and untreated flasks. A period of one half hour was allowed to intervene after the oxygen treatment before transfers were made to the milk tubes, which were then placed in an incubator at 37°C. Four trials were made with dilution tests as indicated in table 3.

Dilution tests indicate some germicidal action in the tubes

TABLE 3

The germicidal and inhibitory action of oxygen on a spore-forming anaerobe as indicated by dilution test

PERIOD OF OXYGEN TREATMENT	TRIAL 1*						TRIAL 2						TRIAL 3						TRIAL 4					
5 minutes	+	+	+	−	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	−
No oxygen	+	+	+	+	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	+
15 minutes	+	+	+	−	−	−	+	+	−	−	−	−	+	+	−	−	−	−	+	+	+	+	+	−
No oxygen	+	+	+	+	−	−	+	+	−	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+
30 minutes	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	−	−	+	+	−	−	−	−
No oxygen	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−

* + = growth; − no growth.

from flasks treated with oxygen for five and fifteen minutes and a rather marked action when treated for a period of thirty minutes. In one case the milk was sterile, and in another case growth occurred in the first dilution as a result of the oxygen treatment for thirty minutes.

The original flasks treated with oxygen showed only a little, gas, and in the case of the thirty-minute treatment, practically none when incubated at 30°C. for twenty-four hours; whereas the corresponding check flasks showed the presence of gas to a marked degree. The oxygen-treated flasks gave a solid marblelike curd and very little if any gas was in evidence even after a period of several days. The action of oxygen on the spore-forming anaerobes, therefore, appears to be both inhibitory and germicidal.

THE EFFECT OF AIR ON THE GASSY FERMENTATION IN SWISS CHEESE

Several experiments were made to determine what effect running air itself through the milk would have on the gassy fermentation. The results of the following experiment are shown in table 4: In cheeses 1 and 2 air was run into milk by means of

TABLE 4

Effect of air on the gassy fermentation in Swiss cheese

NUMBER	QUANTITY OF MILK	QUANTITY OF GAS CULTURE	MANNER OF AERATION	TEMPERATURE OF MILK TREATED	GRADE OF CHEESE*	EFFECT OF AIR†	REMARKS
	lbs.	cc.		cc.			
1	370	1,100	Air blown through coil for 20 minutes	2	No. 1	0	More of a pressler tendency than check
1-1‡	370	1,100		2	F. g		Trace gas edge
2	370	1,000	22 cubic feet of air through	6	No. 2	0	Both cheeses pressler and nissler
2-1‡	370	1,000		6	No. 2		
3	370	1,000	Through aerator twice	3	No. 2	0	Both cheeses pressler and nissler
3-1‡	370	1,000		3	No. 2		
4	370		Through aerator once	6	No. 2	0	Very much overset. Not so good as check
4-1‡	370			6	No. 1 p		
5	370		Through aerator once	6	No. 2	0	Genuine nissler
5-1‡	370			6			The better of the two

* F = fancy (Fancy and No. 1 further divided: g = good; f = fair; p = poor).
† 0 = no improvement.
‡ Check cheese.

a copper coil; in nos. 3, 4, and 5 the air was forced through the milk by means of an especially constructed aerator, the purpose of which was to insure a maximum aeration of the milk. The aerator consisted essentially of a cylinder of two compartments separated from each other by means of a perforated disc of tin

containing 75 small holes through which the milk slowly percolated from the upper to the lower chamber. Beneath the level of the lower chamber the milk was drawn off by a pipe containing a U-shaped bend. Several inches of free space thus existed between the surface of the milk and the disc. Air was forced into the lower chamber by means of a motor-propelled sirocco fan which caused the air to come in contact with the milk dropping from the upper chamber and then to pass through the perforated disc into the milk in the upper chamber. Gas cultures were not used in cheeses 4 and 5 as the milk was of poor quality.

Experiments show that Swiss cheese made from milk treated with air showed no improvement with respect to checking gassy fermentations.

SUMMARY

Ozone and oxygen had a somewhat similar effect in checking gassy fermentation in Swiss cheese occasioned by the introduction of spore-forming anaerobes and did not interfere with the normal functioning of the ripening agents. Within the limits used the temperature did not seem to influence appreciably the effectiveness of these germicides.

Ozone, however, generally imparted such an objectionable flavor to the cheese as to render it unmarketable.

In the case of oxygen some advantage was observed in introducing 20 cubic feet of the gas instead of 10 cubic feet.

In two cases when oxygen was added to milk delivered once a day and the cheese was made upon a commercial scale without the addition of gas-producing organisms, favorable results were observed in checking nissler tendencies. This suggests, at least for this particular factory, that the organisms responsible for abnormal fermentations were anaerobic in character.

The action of oxygen, as indicated by dilution and flask tests, seemed germicidal as well as inhibitory.

In the case of air the oxygen appeared to be diluted too much to exert any favorable influence upon the gassy fermentation.

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THE IMMEDIATE INFLUENCE OF FEEDS UPON THE QUANTITY AND QUALITY OF COW'S MILK

I. THE EFFECT OF GROUND FLAX*

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INTRODUCTION

It is a commonly accepted fact that feeds in general exert no specific effect upon the fat percentage in milk over a long period of time. It is, however, generally agreed that certain feeds will cause a temporary increase in fat percentage which will gradually return to normal even though such feed is continuously administered. Many also believe that certain feeds will stimulate milk production for a short period to a point that cannot be maintained by continuous feeding of such feeds. While these facts are generally known and substantiated by experimental evidence, a search of the literature failed to reveal any evidence as to how soon after the administration of a feed the stimulating effect occurred upon either the quantity or quality of the milk.

If the sudden introduction of a feed into the ration will cause an immediate increase in the fat percentage, or the amount of milk, or both, then it becomes possible to take advantage of this in getting more credit for a cow on semi official test[1] than she actually produced. It is because of this possibility that an investigation was undertaken at the University of Minnesota to determine the immediate effect and the extent to which the quantity and quality of cow's milk might be affected by feeding of certain feeds.

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[1] The present system of semi-official testing consists of a one or two-day test each month by official testing supervisors. The fat percentage found on such days is applied to the monthly milk production for the monthly fat credit. The milk weights found by the official test supervisors serve as a check upon the weights reported by the breeder.

Ground flax, being a feed commonly thought of as being capable of influencing both the quantity and quality of milk, was the first studied, and the results of which study are herein reported.

REVIEW OF LITERATURE

The question of the effect of various fatty feeds upon the quantity and quality of milk is an old one, and was the subject of much of the early investigational work in dairy husbandry. The early investigations were conducted from a standpoint of more or less permanent effect of continuous feeding of fatty feeds upon the quantity and quality of milk. While results of different workers were not uniform, the preponderance of evidence was to the effect that fatty feeds do not permanently affect the fat percentage of milk. Anderson (1) as early as 1899, in reviewing the literature to that time and reporting results of his own investigations, came to that conclusion. Of thirteen investigations reviewed by him, only four were reported as showing an increase in fat due to the feeding of fatty feeds. Morgan and co-workers (2), summarizing the work in 1904, cited four investigators as having secured marked increase in amount of milk and fat percentage; thirteen as having secured only slight increases which were only temporary; and three as having secured no effects from the feeding of fatty foods.

That some investigators secured positive results and others negative results is explained by Stohman and co-workers (3) as being due to the fact that individuality of the animals determines whether or not they will respond. To this may be added that the short experimental periods usually used in the reversal type of experiment would indicate temporary effects as being permanent.

While no investigations reported showed the immediate influence, it is of interest to find that different investigators reported an increase in fat percentage or amount of milk or both as a result of feeding flax or flax products. Lindsey (4), feeding 1.4 pounds digestible oil, daily, in ground flax, increased the fat percentage from 5.0 to 5.56 per cent, which gradually returned to normal in four to five weeks. Einecke (5), working with goats, secured an

increase in milk flow in all cases when 50 grams linseed oil were fed daily, and an increase with some animals and a decrease with others when 30 grams were fed. In all cases the fat percentage rose. Beglarian (6), feeding ground flax observed an increase in the amount of milk but no influence on the fat percentage, with four cows on four experiments of eight days each. Morgan and co-workers (7), feeding 1 gram linseed oil per kg. live weight to two goats and eight sheep reported various results from a decrease in both milk and fat to a very marked increase.

This brief review of the extended literature on the subject is sufficient to show that marked permanent effects upon either the quantity or quality of the milk is not secured by the feeding of flax or flax products but that the temporary effects have often been noted.

THE PROBLEM

As it is fairly well established that no feed exerts a specific influence upon the fat percentage over a long period of time, no attempt was made in this investigation to ascertain over how long a period of time ground flax might exert an influence. This investigation is concerned primarily with the possibility of ground flax exerting a specific influence upon milk or milk-fat production of cows immediately after ingestion. From this standpoint, the following factors were considered:

1. The effect upon the fat percentage and amount of milk.
2. Uniformity of response from time to time.
3. Individual variations. Probable causes and factors that might be correlated with them.
4. Significance of results as applied to semi-official testing.

PLAN OF THE EXPERIMENT

All cows on official test at University Farm during the calendar year were included in the experiment. The official inspection covering two days, with a preliminary milking each month, was conducted in the usual way by a regular official test supervisor. The flax feed period consisted of the forty-eight-hour period immediately following the regular official inspection period.

The tests of the flax feed period were conducted in the same manner and by the same supervisor as official test period.

The ration for the two periods was the same except that on the last feed of the official test period and for each feed of the flax period, one pound of ground flax was substituted for an equal amount of the regular grain mixture. In all other respects the handling and care of the cows for the two periods was identical.

The cows were milked three times daily until the production

TABLE 1

The effect of feeding ground flax upon the fat percentage in milk

COW NUMBER	BREED	NUMBER OF TEST PERIODS	AVERAGE FAT PERCENTAGE		
			Normal period	Flax period	Increase
152	Jersey	12	5.382	5.671	+0.289
144	Jersey	9	5.239	5.484	+0.245
145	Jersey	7	4.621	4.636	+0.015
140	Jersey	7	5.791	5.844	+0.053
141	Jersey	5	6.630	6.831	+0.201
143	Jersey	8	6.298	6.464	+0.166
360	Holstein	9	2.663	2.654	−0.009
376	Holstein	8	3.043	3.087	+0.044
350	Holstein	10	3.058	3.068	+0.010
361	Holstein	7	3.480	3.465	−0.015
355	Holstein	9	2.959	3.134	+0.175
358	Holstein	8	3.320	3.555	+0.235
520	Guernsey	7	5.222	5.250	+0.030
536	Guernsey	6	4.567	4.909	+0.342
526	Guernsey	8	4.837	4.971	+0.134
527	Guernsey	4	5.179	5.368	+0.189
Average			4.518	4.649	+0.131

dropped to the point where the University Farm scale called for a change to two-time milking. Thus when milked three times daily, each cow received three pounds ground flax, and when milked twice daily, each cow received two pounds flax daily.

All cows were fed alfalfa hay, corn silage, beet pulp, and the University Farm test ration consisting of:

Corn.....100 parts
Bran.....100 parts

Ground oats	100 parts
Barley	100 parts
Linseed oil meal	100 parts
Cottonseed meal	50 parts
Corn gluten	50 parts
Salt	9 parts

DISCUSSION OF RESULTS

General effect upon milk and fat percentage. From table 1 it will be seen that the average fat percentage for the 16 cows is 0.131 points or 2.9 per cent higher for the two-day flax periods

TABLE 2

The effect of feeding ground flax upon the two-day milk and fat yield

COW NUMBER	AVERAGE TWO-DAY MILK YIELD			AVERAGE TWO-DAY FAT YIELD		
	Normal period	Flax period	Increase	Normal period	Flax period	Increase
152	52.9	54.3	+1.4	2.86	3.08	+0.23
144	44.3	46.4	+2.1	2.32	2.54	+0.12
145	46.5	48.0	+1.5	2.15	2.22	+0.07
140	36.4	36.1	−0.3	2.11	2.11	0.00
141	16.8	18.3	+1.5	1.11	1.25	+0.14
143	28.9	30.2	+1.3	1.82	1.95	+0.13
360	106.0	108.7	+2.7	2.83	2.89	+0.06
376	115.7	117.6	+1.9	3.52	3.63	0.11
350	93.5	90.1	−3.4	2.86	2.77	−0.09
361	74.0	74.9	+0.9	2.57	2.60	+0.03
355	90.3	87.8	−2.5	2.67	2.75	+0.08
358	80.0	79.8	−0.2	2.66	2.84	+0.18
520	35.9	37.2	+1.3	1.87	1.95	+0.08
536	69.4	70.5	+1.1	3.17	3.46	+0.29
526	51.3	52.8	+1.5	2.48	2.62	+0.14
527	48.0	48.4	+0.4	2.49	2.60	+0.11
Average.....	61.9	62.6	+0.7	2.47	2.58	+0.11

than for the normal feed period. Further analysis reveals that 14 out of the 16 cows increased, and only two decreased the fat percentage when fed ground flax. However, for the two that decreased and four of those that increased the fat percentage, the variation is less than one per cent from the normal feed period. For the rest the effect is appreciable, varying from increases of

1.5 per cent to 7.1 per cent. In four cases the actual increases in fat percentage are more than 0.2, and in one case it is 0.342.

From table 2 it will be seen that on the average the milk production for the two-day flax period is 0.7 pounds or 1.1 per cent more than for the two-day normal feed period. Further study of this table reveals that different cows responded in widely varying degrees. Four cows declined and twelve cows increased in milk production when fed ground flax. The variation is from a decline of 3.6 per cent to an increase of 8.9 per cent. In six cases the increase or decrease is less than 1 per cent from normal, and may be considered as insignificant.

Table 2 also shows the influence of ground flax on the fat percentage and milk combined, and expressed as total fat production for the two-day period. Fourteen cows responded to the feeding of ground flax by increases in fat production varying from 0.9 to 12.1 per cent over the normal feed period. In one case there will be noted a decrease of 0.90 pounds fat or 3 per cent, and in the other case, no effect. The decrease in fat production is due to the depressing influence of flax upon milk flow as there is no appreciable influence upon the fat percentage in this case. Cow 536 was habitually low on the first milking of the official test which was with two exceptions the evening milking, and highest on the second or morning milking. It will be noted that on the flax period both the milk and the fat percentage is higher for the first milking and lower for the second milking than for the normal feed period. After the second milking the fat percentage gradually increases reaching the maximum at the fourth milking.

Figure 1 illustrates graphically the immediate effect of ground flax upon the milk production and fat percentage for three cows. It shows the average amount of milk and fat percentage for each of the six milkings during the experiment for both the normal feed and flax periods. Cow 152 increases both milk production and fat percentage when fed flax. The stimulating effect upon milk production was immediate as the first milking of the flax period was the highest which was eight hours after the first feeding of flax. It will also be noted that the curve for milk production is uniform up to the sixth milking when it drops off

abruptly. Fat percentage is also increased on the first milking but there is a gradual rise up to the fifth milking when the maximum is reached.

Uniformity of response. Figures 2, 3, and 4 illustrate what may be expected in the way of difference of response by different cows from month to month. Figure 2 is the graph, by months,

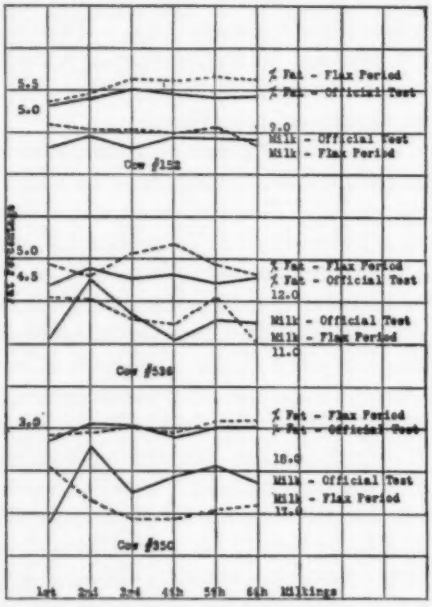

FIG. 1. SHOWING THE IMMEDIATE EFFECT OF GROUND FLAX UPON FAT PERCENTAGE AND AMOUNT OF MILK ON THREE COWS

The corresponding milkings of the flax feed period and the normal feed period are compared.

of the production record of cow 152 for both normal feed and flax periods. It shows that both the amount of milk and the fat percentage was stimulated fairly uniformly with the exception of the first, tenth and eleventh months of the lactation period. The increase in fat percentage and the decrease in amount of milk during the flax period of the first month may be attributed

to the fact that she was in oestrum during the flax period. The higher fat percentages for the official test period of the tenth and eleventh month is unaccounted for. It may be noted, however, that the fat percentages for these months are unusually high on the official test period and that the flax period presents a smoother curve.

Cow 350 showed no response to feeding of ground flax as far as fat percentage is concerned but showed a rapid decline in milk, reaching the minimum at the third milking. For some unknown

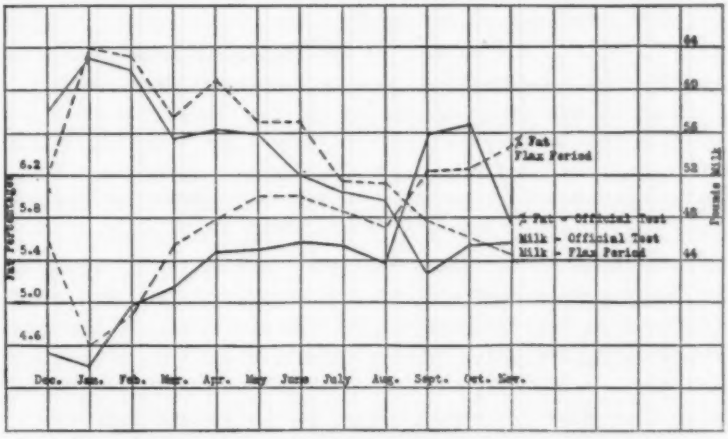

FIG. 2. COMPARISON BY MONTHS OF FAT PERCENTAGE AND AMOUNT OF MILK FOR FLAX PERIOD AND NORMAL FEED PERIOD FOR COW 152

reason, this cow averaged low on the first milking of the normal feed period and high on the second milking. In the other cases where ground flax had a depressing influence upon milk production, it stimulated fat percentage sufficiently to account for an increase in total fat production.

Figure 3 illustrates the uniformity of response to the depressing influence of ground flax upon the milk production of cow 350, and varied influence upon the fat percentage. With the exception of one month, the milk production was lower each time for the flax period than for the official test period.

Figure 4 illustrates a variance in response to the feeding of flax both for milk production and fat percentages. On the aver-

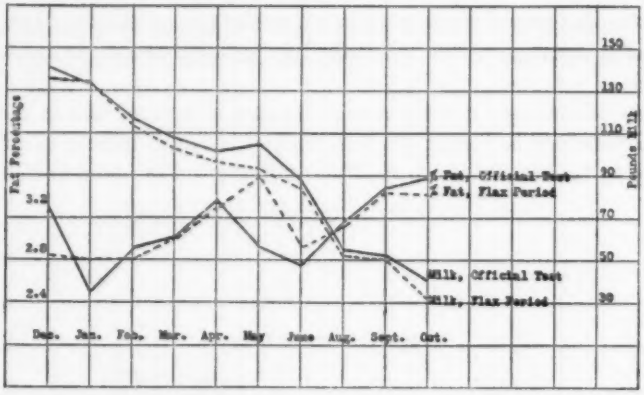

FIG. 3. COMPARISON BY MONTHS OF FAT PERCENTAGE AND AMOUNT OF MILK FOR FLAX PERIOD AND NORMAL FEED PERIOD OF COW 350

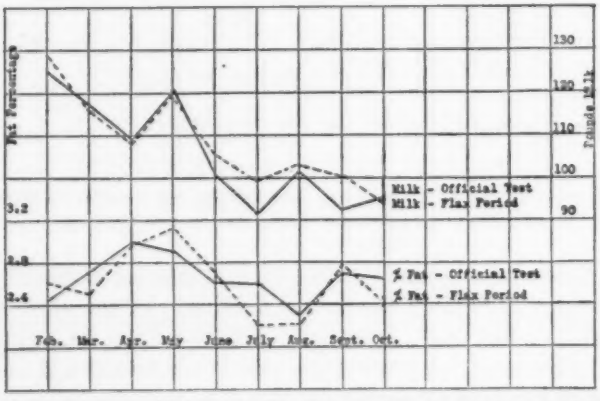

FIG. 4. COMPARISON BY MONTHS OF FAT PERCENTAGE AND AMOUNT OF MILK FOR FLAX PERIOD AND NORMAL FEED PERIOD FOR COW 360

age flax caused a slight increase in both milk production and fat percentage but a variable response from month to month.

Individual variations and probable causes. One of the signifi-

cant results of this investigation is that individual animals vary in the way and degree that they respond to the feeding of ground flax. From the data herein presented, we would not be warranted in concluding that ground flax has a depressing effect upon the fat percentage of some cows but can conclude that with some it has no effect and that with others the fat percentage is stimulated to varying degrees. With milk production, the feeding of ground flax may have a depressing effect on some cows, no influence on others, and a stimulating influence on still others. Wherever there was either a marked depressing or stimulating effect on either the average milk or fat production, such influence was uniform each time the animal was fed ground flax.

Neither stimulating nor depressing influences of flax feeding could be correlated with breed, capacity for milk production, normal fat percentage of milk, season of year or period of the lactation. As to whether or not an animal will respond by an increase or decrease in either the amount or the fat percentage of milk through the feeding of ground flax depends upon some unknown factor peculiar to the individual. From the data herein presented, it would appear that separate factors are involved for milk and fat percentage as a depressing effect upon milk production may or may not be accompanied by a stimulating effect upon fat percentage or vice versa.

As an increase in body temperature is usually believed to cause an increase in the fat percentage of the milk, it was thought possible that the ingestion of the relatively large amounts of ground flax might cause metabolic disturbances that in turn might increase the body temperature. Temperatures were then taken for three consecutive months at eight hour intervals for both normal and flax feed periods. When analysis of the data revealed no increases in body temperature from the feeding of flax, this particular phase of the work was discontinued.

Significance of results as applied to semi-official testing. As the average fat percentage credited a cow on semi-official test depends upon the results secured by the official test supervisor on the monthly one or two-day inspections, it is evident that anything that will immediately and temporarily increase the test will credit

the cow with more butterfat than she is entitled to. If milk production can be consistently increased by the specific effect of a feed during the one or two-day official monthly inspection, the owner can pad the milk reports so as to get more credit than a cow is entitled to, and such padding cannot be detected from inspection of the milk reports. Table 3 shows that by taking advantage of the temporary stimulating effects of ground flax

TABLE 3

The effect on official test record of applying flax period fat percentage and milk yield during experimental period

COW NUMBER	INCREASE OF OFFICIAL TEST BUTTERFAT RECORD MADE POSSIBLE BY APPLICATION OF:			
	Flax period fat percentage		Combined milk and fat percentage	
	pounds	*per cent*	*pounds*	*per cent*
152	24.66	4.6	43.42	8.1
144	13.21	3.9	17.68	5.3
145	−0.10	0.0	8.75	3.6
140	2.16	0.9	0.00	0.0
141	2.44	2.9	10.03	12.1
143	6.78	3.0	16.46	7.3
360	−0.50	−0.1	7.82	2.0
376	7.44	1.3	12.99	3.0
350	0.84	0.0	−13.18	−3.0
361	1.63	0.6	2.29	0.9
355	17.72	4.7	9.65	2.6
358	24.04	7.6	21.68	6.8
520	2.17	1.0	8.58	4.3
536	22.27	7.2	28.28	9.2
526	8.35	2.7	16.95	5.7
527	8.01	5.0	7.23	4.5
Average.....		2.8		4.5

upon both the amount and fat percentage of milk 14 out of 16 cows would have been credited with more than their actual butterfat production, and only one would have received less credit. The increases in total butterfat production varied from 0.9 per cent to 12.1 per cent, while the one decrease amounted to 3 per cent.

Cow 152 was credited officially with 536.4 pounds fat. Had advantage been taken of the stimulation caused by the feeding

of ground flax, and the results secured on the flax periods applied instead of the official tests, she would have been credited with 579.8 pounds fat during the same period. Calculated on this same basis, cow 536 shows an increase of 28.28 pounds fat for a period of six months.

With some cows, therefore, advantage can be taken of the stimulating effect of ground flax to secure more credit than is actually due on semi-official test. However, the owner must know how the cow is going to respond in order to do this as the irregularity of response of some cows from month to month will often give the actual milk report the appearance of having been padded when ground flax is fed for the official test period.

CONCLUSIONS

From the results of this investigation it may be concluded that:

1. The response of animals to the feeding of ground flax depends upon unknown individual factors. Different animals respond in different ways and to different degrees, and such differences are not correlated with season of the year, breed, amount of yearly milk production or normal fat percentage of the milk.

2. When ground flax is fed at the rate of three pounds daily the majority of cows will show an increase in fat percentage. Such increases ranged from no effect to 7.1 per cent and averaged 2.9 per cent.

3. The slight decrease in fat percentage in two cases out of sixteen cows will not warrant the conclusion that ground flax may cause a decrease in fat percentage but rather that in some cases it has no appreciable effect.

4. The feeding of ground flax will in the majority of cases cause an increase in amount of milk averaging 1.1 per cent but going up to 8.9 per cent, but ground flax may also have a depressing influence upon the milk production with a few cows.

5. On the average the combined influence of flax upon amount of milk and fat percentage increased the total fat production 4.5 per cent. This influence varied from a decrease of 3.0 per cent to an increase of over 12.0 per cent.

6. With cows showing appreciable responses, on the average, the response was uniform from month to month, making it possible to secure more than actual credit for cows on official test.

7. The response, whether an increase or decrease, was immediate with most cows, appearing in most cases within eight hours after the first feeding. This makes detection of such feeding practices impossible from inspection of official test record.

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DAIRY NOTES

ADULTERATION OF MILK

In most of the books which treat of the subject, the formulae given for calculation of the percentages of added water and fat removed in cases of adulterated milk are as follows:

$$p = 100(1 - f/F); \; w = 100(1 - n/N)$$

f and n are respectively the percentages of fat and non-fatty solids found, F and N the percentages known or assumed to have been originally present, w the percentage of added water and p the percentage of the fat removed.

The first of these two formulae is not exact; and should be:

$$p = 100\left(1 - \frac{f}{F}\right) \times \frac{100}{100 - f}$$

In any case it fails entirely if the milk has been watered. The second is exact only if none of the fat has been removed. This is generally recognised and some of the books give other formulae to be used in special cases, i.e., when the milk has been both skimmed and watered.

These special formulae are not regarded with much favour by analysts. In some cases they are too complicated; in others they are obviously inaccurate. If it were exact, the formula for fat removed should give the true result whether the amount of added water were large or small or even if it were zero. Similarly, the formula for added water would be independent of the amount of fat removed.

In other words, if they were exact, these formulae for the special case of milk that has been both skimmed and watered would be perfectly general; they would be applicable in every case and no others would be required. It is the purpose of this article to show that such expressions can be found, that they are quite simple and that those previously referred to can therefore be dispensed with. The method by which they are derived is briefly outlined below.

If 100 grams of milk contain 4 grams of fat and 9 grams of non-fatty solids and 100 grams of water be added to it there will be 200 grams of mixture. This will contain 50 per cent of added water and the original amounts, but only half the percentages, of the other ingredients. If

now 1 gram (25 per cent) of the fat be removed there will be left 199 grams of skimmed and watered milk and this will contain 3 grams (= 1.5075 per cent) of fat and 9 grams (= 4.5226 per cent) of non-fatty solids.

In general, if f' and n' be the percentages of fat and non-fatty solids, respectively, in the skimmed and watered milk and the other symbols as before, then:

$$f = \frac{F(100 - w)}{100}; \; f' = \frac{100f(100 - p)}{10{,}000 - pF}$$

$$n = \frac{N(100 - w)}{100}; \; n' = \frac{10{,}000n}{10{,}000 - pf}$$

Substituting the values of f and n in the second equation in each case this gives:

$$f' = \frac{100F(100 - w)\ (100 - p)}{1{,}000{,}000 - pF(100 - w)}; \; n' = \frac{10{,}000N(100 - w)}{1{,}000{,}000 - pF(100 - w)}$$

As each of these equations involves both p and w, these factors cannot be evaluated from either; but if they are treated as a pair of simultaneous equations, expressions can be found for both p and w. The result is as follows:

$$p = 100\left\{1 - \frac{Nf'}{n'F}\right\}; \; w = 100\left\{1 - \frac{100n'}{N(100 - f') + n'F}\right\}$$

If 3 per cent of fat and 8.5 per cent of non-fatty solids be taken as the original amounts of these ingredients and f and n be the percentages found, the formulae become:

$$p = 100\left\{1 - \frac{8.5f}{3n}\right\}; \; w = 100\left\{1 - \frac{100n}{8.5(100 - f) + 3n}\right\}$$

These formulae are not unduly complicated. They are exact and are therefore applicable whether the milk is both skimmed and watered or adulterated in one way only. The formula for added water is new; at all events the author has not seen it given previously.

The formula for fat removed is not new; it was given by Bohmlander (Chemiker-Zeitung, vol. xvii) in 1893. Exception may be taken to it on the ground that it is based on the alternation of the ratio F/N, which of course is not affected by addition of water, and that the legislation

under which action is taken in such cases does not mention and therefore does not sanction the use of this or any other "ratio."

It is evident, however, from the manner in which the formula is derived that it is a legitimate mathematical inference from the given premises. It is therefore, in that respect, just as valid as the formula for fat removed which is applicable only when it is known or assumed that no water has been added. In fact, under these circumstances, it gives the same result.

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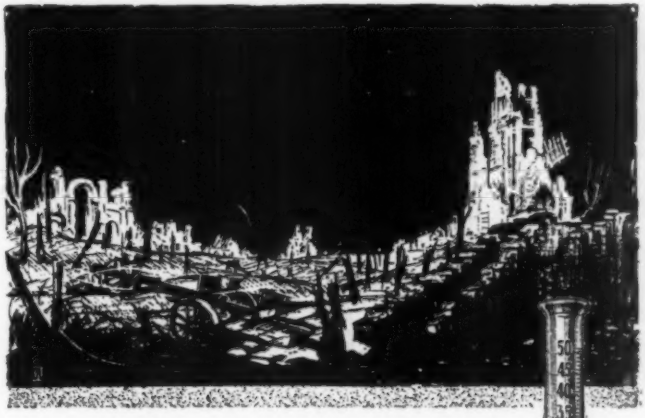
University College, Reading, England.



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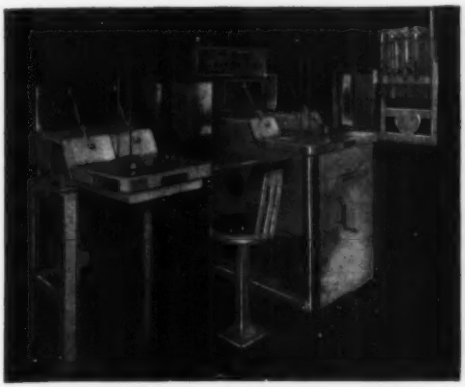

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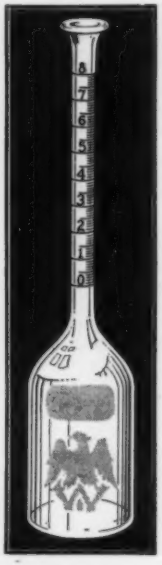

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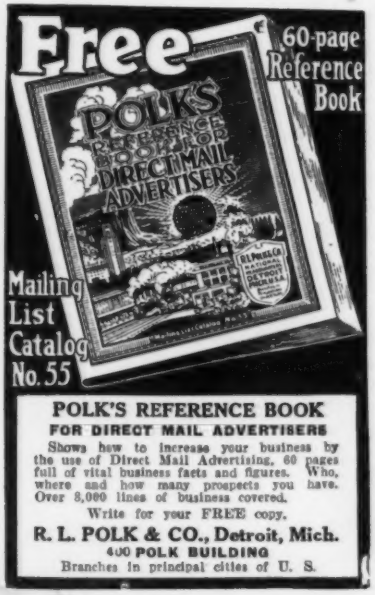

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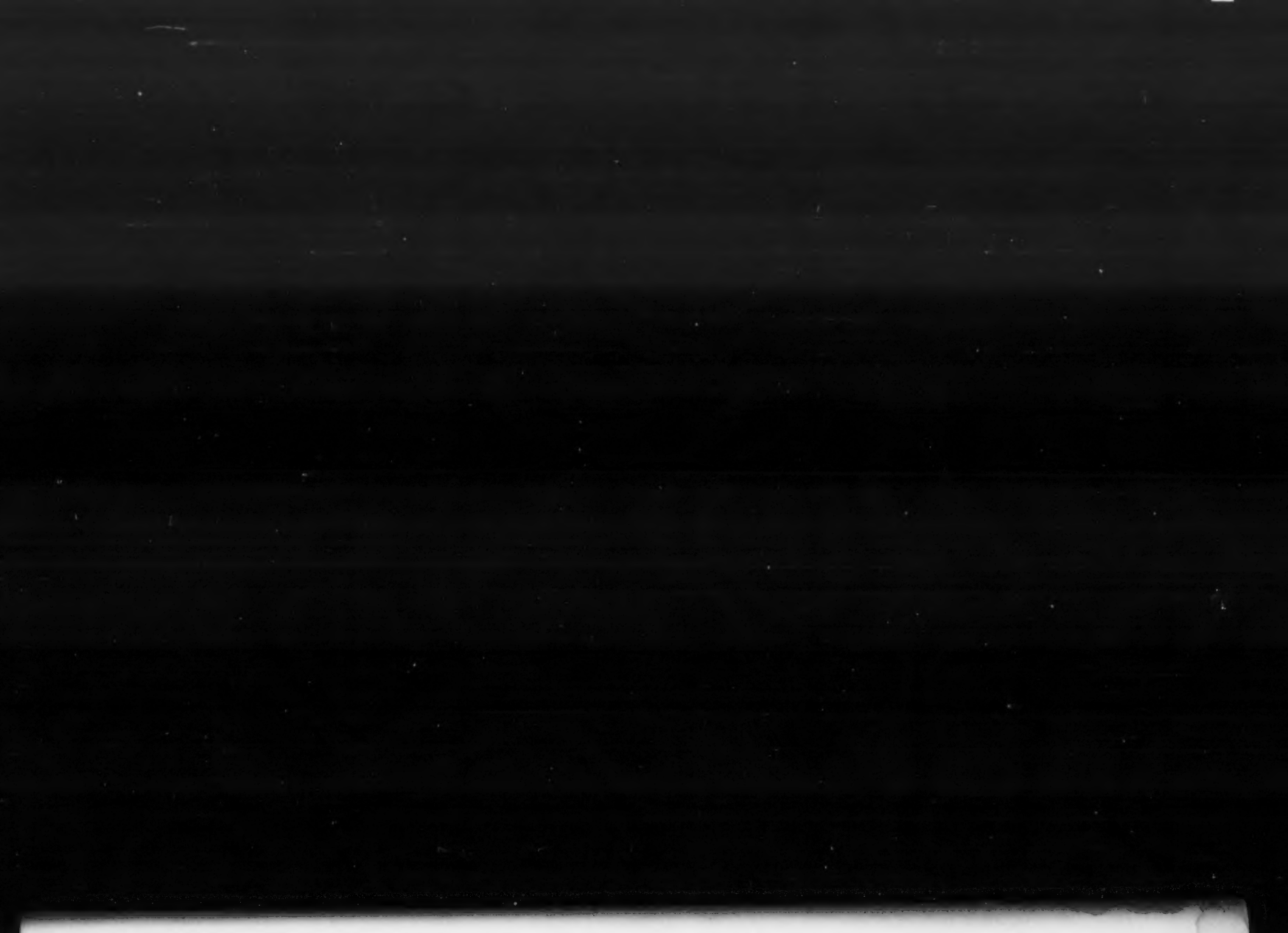

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